WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, July 22, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	postranlat$.ti.	0
☐	L2	postranslat$.ti.	0
☐	L3	posttranslat$.ti.	2
☐	L4	post-translat$.ti.	7
☐	L5	post-translat$.clm. or posttranslati$.clm. not l1 not l2 not l3 not l4	91
☐	L6	L5 and covalent$	56

END OF SEARCH HISTORY

Search Results - Record(s) 1 through 5 of 5 returned.

L29: Entry 1 of 5 File: USPT Jul 17, 2001

US-PAT-NO: 6261793
DOCUMENT-IDENTIFIER: US 6261793 B1

TITLE: RAS converting endoprotease (RCE) and methods

DATE-ISSUED: July 17, 2001

US-CL-CURRENT: 435/15; 435/193, 435/4, 436/63, 436/64, 530/350

INT-CL: [07] C12 Q 1/48, C12 N 9/10, G01 N 33/48, C07 K 14/435

L29: Entry 2 of 5 File: USPT Mar 14, 2000

US-PAT-NO: 6037136
DOCUMENT-IDENTIFIER: US 6037136 A

TITLE: Interactions between RaF proto-oncogenes and CDC25 phosphatases, and uses related thereto

DATE-ISSUED: March 14, 2000

US-CL-CURRENT: 435/7.4; 435/15, 435/21, 435/7.8

INT-CL: [07] G01 N 33/573

L29: Entry 3 of 5 File: USPT Nov 16, 1999

US-PAT-NO: 5985589
DOCUMENT-IDENTIFIER: US 5985589 A

TITLE: Lipid kinase

DATE-ISSUED: November 16, 1999

US-CL-CURRENT: 435/15; 435/194, 435/252.3, 435/320.1, 435/69.1, 435/69.2, 435/7.7, 530/350, 536/23.2, 536/23.5

INT-CL: [06] C12 Q 1/48, C12 N 9/12, C12 P 21/06, C07 H 21/04

L29: Entry 4 of 5 File: USPT Jul 7, 1998

US-PAT-NO: 5776675
DOCUMENT-IDENTIFIER: US 5776675 A

TITLE: Identification of compounds modulating protein/cell membrane association

DATE-ISSUED: July 7, 1998

US-CL-CURRENT: 435/6; 435/254.21, 435/320.1, 435/325, 530/350

INT-CL: [06] C12 Q 1/68, C12 N 1/19, C12 N 15/79, C07 K 14/00

L29: Entry 5 of 5 File: USPT Jun 11, 1996

US-PAT-NO: 5525490
DOCUMENT-IDENTIFIER: US 5525490 A

TITLE: Reverse two-hybrid method

DATE-ISSUED: June 11, 1996

US-CL-CURRENT: 435/29; 435/254.21, 536/23.7

INT-CL: [06] C12 Q 1/02, C12 N 15/81, C12 N 15/11

Previous Page Next Page

Derwent Accession: 2003-361860

Compositions and methods for monitoring the modification of modification dependent binding partner polypeptides

Inventor: Roger Craig, INV
Correspondence Address: PALMER & DODGE, LLP KATHLEEN M. WILLIAMS, 111 HUNTINGTON AVENUE, BOSTON, MA, 02199, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20020197606	A1	20021226	US 2001770102	20010125
Provisional				US 60-179283	20000131

Fulltext Word Count: 35830

Summary of the Invention:

...are brought into physically close proximity with one another can exhibit fluorescence resonance energy transfer (" **FRET** "). The invention of WO97/28261 takes advantage of that discovery to provide tandem fluorescent protein constructs in which two fluorescent protein labels capable of exhibiting **FRET** are coupled through a linker to form a tandem construct. In the assays of the Tsien et al. application, protease activity is monitored using **FRET** to determine the distance between fluorophores controlled by a peptide linker and subsequent hydrolysis thereof...which are labeled with fluorescent or non-fluorescent labels (protein and chemical). In the invention, **FRET** , fluorescence correlation spectroscopy, fluorescence anisotropy, fluorescence polarization, monomer:excimer fluorescence, or other techniques indicate the...molecules. Detecting can then comprise monitoring the presence or absence of fluorescent resonance energy transfer (**FRET**).

[...The detecting can then comprise monitoring the presence or absence of fluorescent resonance energy transfer (**FRET**).

[...

...tagged binding partners under conditions which promote binding or dissociation of said one or more **immobilized** binding partner polypeptides with said one or more tagged **binding partners** .

0005183881 **IMAGE Available
Derwent Accession: 2003-503362

Surface plasmon enhanced illumination system

Inventor: Peter Stark, INV
Dale Larson, INV
Correspondence Address: CHARLES G. CALL, 68 HORSE POND ROAD, WEST YARMOUTH, MA, 02673-2516, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20030036204	A1	20030220	US 2002218928	20020814
CIP	PENDING			US 2001981280	20011016
Provisional				US 60-312214	20010814

Fulltext Word Count: 28177

Summary of the Invention:

...locations of proteins below the Rayleigh limit. They include: Confocal Microscopy, Fluorescence Resonance Energy Transfer (**FRET**), Atomic Force Microscopy (AFM), Near-Field Scanning Optical Microscopy (NSOM), Harmonic Excitation Light-Microscopy (HELM...0007] Fluorescence Resonance Energy Transfer (**FRET**) can provide exquisite resolution of single chromophores. The resonance occurs when one fluorophore in an...

...radiative decay or dipole-dipole interaction. The Forster distance, essentially, is the threshold at which **FRET** will no longer exist for a given pair. Typically the Forster distance is between 3 and 6 nm [see Pollok & Heim "Using GFP in **FRET** -based Applications" Trends in Cell Biology 9 pp57-60 (1999...between a light source and a detector may be used to analyze ligands that are **immobilized** on the surface barrier. The ligands' **binding partners** bind to the ligands **immobilized** on the illuminated surface and, as that occurs, or after it has occurred, a shift...

Description of the Drawings:

...0057]FIG. 33 is a schematic diagram of a sensor for analyzing ligands which are **immobilized** at the conductive surface of a hole array and their **binding partners** ;

[

0005431922 **IMAGE Available
Derwent Accession: 2003-901578

Molecular interaction assays on a solid surface

Inventor: Nelson, Bryce, INV
Strother, Todd, INV
Correspondence Address: Tanya A. Arenson MEDLEN & CARROLL, LLP, Suite 350 101 Howard Street, San Francisco, CA, 94105, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20030211480	A1	20031113	US 2002141611	20020508

Fulltext Word Count: 21237

Description of the Invention:

...RNA expression is detected using any suitable method including, but not limited to SPR, fluorescence, **FRET** , electrophoretic methods, and RT-PCR...

...DNA duplexes, in some embodiments, hybridization of the nascent RNA to a self-complementary DNA **FRET** probe is used to detect the RNA molecule. The RNA will out-compete the self complementary DNA for hybridization. In some embodiments, the **FRET** quenching dye is designed within the hybridization region, and the detected fluor is linked near...invention are utilized in high throughput or combinatorial assays. For example, to query multiple first **binding partners** against multiple second **binding partners** , the first fusion partner is **immobilized** , and the surface array is monitored for interaction of the first and second fusion

```
Set     Items   Description
S1     129256   (POSTTRANSLAT? OR (POST (2N) TRANSLAT?) OR PHOSPHORY? OR A-
             CYLA? OR GLYCOSYLAT? OR UBIQUITINA? OR PRENYLA? OR SENTRINIZ?
             OR RIBOSYLAT?)
S2      48015   FRET OR (ENERGY (2N) TRANSFER?)
S3        353   S1 (100N) S2
S4        341   S3 AND (PEPTIDE? OR POLYPEPTIDE? OR PROTEIN? OR AMINO?)
S5        335   S4 AND (PEPTIDE? OR POLYPEPTIDE? OR PROTEIN?)
S6          1   S4 AND IMMOBLI? AND COVALENT?
S7        180   S1 (25N) S2
S8         51   S7 (25N) (METHOD? OR PROCESS?)
S9       1810   POLYPEPTIDE? (3N) PAIR?
S10        27   S9 (10N) IMMOBIL?
?t s10/3,kwic/all
```

First Hit

Generate Collection

L21: Entry 4 of 6 File: DWPI Mar 25, 2004

DERWENT-ACC-NO: 2002-575157
DERWENT-WEEK: 200422

TITLE: Selecting nucleic acid processing enzyme e.g., replicase by selecting nucleic nucleic acids encoding gene products, in which nucleic acid and activity of encoded gene product are linked by compartmentalization

Basic Abstract Text (7):
USE - (M1) is useful for selecting (M2) an agent capable of modifying the activity of a NAP enzyme. Preferably the method is used for selecting a promoter of NAP enzyme activity, e.g. a kinase or a phosphorylase, which is capable of acting on the the NAP enzyme to modify its activity, a polypeptide involved in a metabolic pathway, which has an end product a substrate which is involved in a NA processing reaction, a polypeptide capable of producing a substrate or consuming an inhibitor in a NA processing reaction, or a polypeptide capable of modifying a nucleotide primer or nucleoside triphosphate substrate used in NA processing reaction such that its 3' end becomes extendible, or a substrate portion appended to the nucleotide primer or nucleoside triphosphate is modified such as to allow detection or capture of product appendage of the incorporated nucleotide primer or nucleoside triphosphate. (M1) is also useful for selecting (M3) a pair of polypeptides capable of stable interaction and detecting processing of at least one of the first and second NA by the NAP enzyme.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/22869 A2

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/GB01/04108

(22) International Filing Date:
13 September 2001 (13.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0022458.4	13 September 2000 (13.09.2000)	GB
60/283,771	13 April 2001 (13.04.2001)	US
60/285,501	20 April 2001 (20.04.2001)	US

(71) Applicant *(for all designated States except US)*: **MEDICAL RESEARCH COUNCIL** [GB/GB]; 20 Park Crescent, London WN1 4AL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HOLLIGER, Philipp** [CH/GB]; MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (GB). **GHADESSY, Farid** [GB/GB]; MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (GB).

(74) Agents: **MASCHIO, Antonio** et al.; D Young & Co, 21 New Fetter Lane, London EC4A 1AD (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/22869 A2

(54) Title: DIRECTED EVOLUTION METHOD

(57) Abstract: We describe a method of selecting an enzyme having replicase activity, the method comprising the steps of: (a) providing a pool of nucleic acids comprising members each encoding a replicase or a variant of the replicase; (b) subdividing the pool of nucleic acids into compartments, such that each compartment comprises a nucleic acid member of the pool together with the replicase or variant encoded by the nucleic acid member; (c) allowing nucleic acid replication to occur; and (d) detecting amplification of the nucleic acid member by the replicase. Methods for selecting agents capable of modulating replicase activity, and for selecting interacting polypeptides are also disclosed.

First Hit

Generate Collection

L21: Entry 5 of 6 File: DWPI Aug 31, 2000

DERWENT-ACC-NO: 2000-572100
DERWENT-WEEK: 200207

TITLE: Measuring different enzyme activities simultaneously by contacting with a binding domain and a binding partner having a site post-translationally modifiable by the enzyme and measuring interaction between them

Basic Abstract Text (5):
(3) a set of two or more polypeptide pairs (IV), each pair comprising a BD and BP, comprising a site subject to post-translational modification by an enzyme where modification of the site by the enzyme affects the BD and BP interaction, and a detectable label on the BD and BP, when they interact a detectable physical characteristic of one or both labels is altered, for each pair the enzyme which affects the interaction between BD and BP is different and the interaction can be distinguished from that of the other pairs;

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/00 — A2

(11) International Publication Number: **WO 00/50630**

(43) International Publication Date: 31 August 2000 (31.08.00)

(21) International Application Number: PCT/GB00/00663

(22) International Filing Date: 25 February 2000 (25.02.00)

(30) Priority Data:

9904401.8	25 February 1999 (25.02.99)	GB
9904392.9	25 February 1999 (25.02.99)	GB
9904393.7	25 February 1999 (25.02.99)	GB
9904398.6	25 February 1999 (25.02.99)	GB
9904395.2	25 February 1999 (25.02.99)	GB
9904407.5	25 February 1999 (25.02.99)	GB
0000771.6	13 January 2000 (13.01.00)	GB

(71) Applicant *(for all designated States except US)*: FLUORESCIENCE LIMITED [GB/GB]; P.O. Box 439, Leeds LS2 9UN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: COLYER, John [GB/GB]; Elfordlea, Mill Lane, Bardsey LS17 9AN (GB). CRAIG, Roger, KingdoN [GB/GB]; Jubilee House Farm, Spen Green, Smallwood, Cheshire CW11 2XB (GB).

(74) Agents: MASCHIO, Antonio et al.; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: ASSAY FOR MEASURING DIFFERENT ENZYME ACTIVITIES SIMULTANEOUSLY

(57) Abstract

A method is provided for measuring simultaneously the activity of a first enzyme and a second enzyme in a system which method comprises: (a) contacting a first binding domain and a first binding partner thereof with said first enzyme and contacting a second binding domain and a second binding partner thereof with said second enzyme; wherein (i) the first binding domain and/or binding partner comprise a site subject to post-translational modification by the first enzyme; (ii) modification of the site by the first enzyme affects the interaction between the first binding partner; (iii) the second binding domain and/or binding partner comprise a site subject to post-translational modification by the second enzyme; and (iv) modification of the site by the second enzyme affects the interaction between the second binding domain and second binding partner; and (b) measuring the interaction between the first binding domain and the first binding partner and measuring the interaction between the second binding domain and the second binding partner.

Refine Search

Search Results -

Terms	Documents
(L3 or L4 or L7 or L9 or L8 or L10 or L11 or L12 or L13).clm. and L27	72

Database:
US Pre-Grant Publication Full-Text Database
US Patents Full-Text Database
US OCR Full-Text Database
EPO Abstracts Database
JPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search: L29

Refine Search

Recall Text | Clear | Interrupt

Search History

DATE: Thursday, July 22, 2004 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND			
L1	posttranslation$.ti.	6	L1
L2	posttranslation$.ab.	34	L2
L3	posttranslation$.clm.	27	L3
L4	post-translation$.clm.	271	L4
L5	post-translation$.ab.	619	L5
L6	post-translation$.ti.	85	L6
L7	phosphoryl$.ti,ab,clm.	12531	L7
L8	acyla$.ti,ab,clm.	46279	L8
L9	glycosyla$.ti,ab,clm.	5002	L9
L10	ubiquitina$.ti,ab,clm.	211	L10
L11	prenyla$.ti,ab,clm.	283	L11
L12	sentiniza$.ti,ab,clm.	1	L12

L13	adpribosyla$.ti,ab,clm. or adp-ribosyla$.ti,ab,clm.	148	L13
L14	(l1 or l2 or l3 or l4 or l5 or l6 or l7 or l8 or l9 or l10 or l12 or l11 or l13)	64181	L14
L15	L14 and (immobli$.clm. or attach$.clm. or coupl$.clm. or bound.clm. or conjugat$.clm. or substrate.clm. or solid.clm. or surface.clm. or fret.clm. or plasmon.clm.)	7870	L15
L16	L15 and (first and second).clm.	1119	L16
L17	L14 same (first and second)	836	L17
L18	(peptide or sequence or polypeptide or protein$ or poly-peptide or amino)	1840929	L18
L19	polypeptide near2 pair	1242	L19
L20	L19 same covalent$	51	L20
L21	L19 same l14	6	L21
	DB=USPT,PGPB,JPAB,EPAB; PLUR=YES; OP=AND		
L22	(US-20030104504-A1| WO-9911774-A1| AU-9888727-A| GB-2342652-A| NO-200000901-A| EP-1007653-A1| JP-2001514849-W| GB-2342652-B| US-20030027220-A1| US-20030100037-A1)![pn]	1	L22
L23	(US-20030104504-A1| WO-9911774-A1| AU-9888727-A| GB-2342652-A| NO-200000901-A| EP-1007653-A1| JP-2001514849-W| GB-2342652-B| US-20030027220-A1| US-20030100037-A1)![pn]	1	L23
L24	(US-20030104504-A1| WO-9911774-A1| AU-9888727-A| GB-2342652-A| NO-200000901-A| EP-1007653-A1| JP-2001514849-W| GB-2342652-B| US-20030027220-A1| US-20030100037-A1)![pn]	1	L24
L25	(WO-9911774-A1)![pn]	1	L25
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND		
L26	l14.clm. and fret.clm.	27	L26
L27	fret.clm. or (energy near2 transfer$).clm.	7613	L27
L28	L27 and (covalent$ or l14).clm.	241	L28
L29	(l3 or l4 or l7 or l9 or l8 or l10 or l11 or l12 or l13).clm. and l27	72	L29

END OF SEARCH HISTORY

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, July 22, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	postranlat$.ti.	0
☐	L2	postranslat$.ti.	0
☐	L3	posttranslat$.ti.	2
☐	L4	post-translat$.ti.	7
☐	L5	post-translat$.clm. or posttranslati$.clm. not l1 not l2 not l3 not l4	91
☐	L6	L5 and covalent$	56
☐	L7	L5 and covalent$.clm.	10
☐	L8	antiprenyl$ or antisentriniz$ or antiubiquitin$ or antiglycosyla$ or antiadpribosy$	11
☐	L9	(antagonist or inhibitor or blocker or modulator or inactivator).clm.	38779
☐	L10	L9 and (method or process).clm.	23344
☐	L11	phosphoryla$.clm.	1464
☐	L12	kinase.clm.	2500
☐	L13	acylation.clm.	801
☐	L14	glycosylat$.clm.	1026
☐	L15	ubiquitina$.clm.	27
☐	L16	prenyla$.clm.	49
☐	L17	sentriniz$.clm.	0
☐	L18	sentriniz$	2
☐	L19	adpribosyl$.clm. or adp-ribosyla$.clm.	19
☐	L20	l10 and (l11 or l12 or l13 or l14 or l15 or l16 or l17 or l18 or l19)	640
☐	L21	l9 same (l11 or l12 or l13 or l14 or l15 or l16 or l17 or l18 or l19)	462
☐	L22	(L21 or l20) and immobil$.clm.	13
☐	L23	('5565326' |'6251621' |'6207391' |'6548266' |'5221736' |'5744313' |'5834216' |'6710026' |'6682898' |'6667179' |'6514689' |'6074852')!.PN.	12
☐	L24	(ras or raf or mapkinase).clm.	3594
☐	L25	L24 and l9	101
☐	L26	L25 and l10	91
☐	L27	l26 and ras!	49
☐	L28	l26 and ras!.clm.	41

☐	L29	('6037136' |'6261793' |'5776675' |'5525490' |'5985589')!.PN.	5

END OF SEARCH HISTORY

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 13 of 13 returned.

☐ 1. Document ID: US 6710026 B1

L22: Entry 1 of 13 File: USPT Mar 23, 2004

DOCUMENT-IDENTIFIER: US 6710026 B1
TITLE: Protein domains in the hepatic glycogen-targetting subunit of protein phosphatase 1 and methods of making and using the same

CLAIMS:

1. A pharmaceutical composition comprising an inhibitor compound which is capable of of blocking the interaction of phosphorylase a with the glycogen-targeting subunit (G.sub.L) of protein phosphatase 1, together with a pharmaceutically acceptable excipient or carrier wherein the inhibitor compound comprises a polypeptide having SEQ ID. NO: 1 or a fragment thereof which is capable of binding phosphorylase a.

3. A method of identifying an inhibitor compound which is capable of blocking the interaction of phosphorylase a with the glycogen-targeting subunit of protein phosphatase 1 comprising; providing a polypeptide comprising SEQ ID. NO: 1 or fragment thereof which binds phosphorylase a; providing a test compound; and comparing the binding of the polypeptide by phosphorylase a in the presence and absence of the test compound; an inhibitor being identified by reduced binding of the polypeptide in the presence of the test compound.

4. A method as claimed in claim 3 wherein the phosphorylase a is labelled; the polypeptide is immobilised on a support; and the binding of phosphorylase a to the polypeptide is determined by measuring the amount of label bound to the support.

5. A method as claimed in claim 4 wherein phosphorylase a is labelled with a label selected from digoxigenin, .sup.32 P or .sup.33 P.

6. A method of reducing the blood glucose level of a mammalian animal comprising administering a therapeutically effective amount of a compound which is capable of blocking the interaction of phosphorylase a with the glycogen-targeting subunit G.sub.L of protein phosphatase 1, wherein the compound comprises SEQ ID. NO: 1 or a fragment thereof which is capable of binding phosphorylase a.

7. A method as claimed in claim 6 wherein the mammalian animal is a human.

8. A method of blocking the interaction of phosphorylase a with the glycogen-targeting subunit (G.sub.L) of protein phosphatase 1 comprising: contacting phosphorylase a with a compound in an amount effective to block the interaction of the phosphorylase a with the glycogen-targeting unit (G.sub.L) of protein phosphatase 1 wherein the compound is a polypeptide comprising SEQ ID NO:1 or a fragment thereof which is capable of binding phosphorylase a.

9. The method according to claim 8, wherein the compound is administered to a subject having a disorder associated with higher than normal blood glucose levels.

10. The method according to claim 9 wherein the disorder is selected from type I or type II diabetes.

11. The method according to claim 8 wherein the polypeptide increases the activity of hepatic glycogen synthase.

12. A compound which is capable of blocking the interaction of phosphorylase a with the glycogen-targeting subunit (G.sub.L) of protein phosphatase 1, wherein the compound comprises a polypeptide having SEQ ID. NO: 1 or a fragment thereof which is capable of binding phosphorylase a.

☐ 2. Document ID: US 6682898 B2

L22: Entry 2 of 13 File: USPT Jan 27, 2004

DOCUMENT-IDENTIFIER: US 6682898 B2
TITLE: High-throughput screening assays for modulators of STAT4 activity

CLAIMS:

1. A method of screening for modulators of STAT4 binding to a STAT4 receptor, the method comprising: incubating a reaction mixture comprising a STAT4 polypeptide, a potential binding modulator, and a receptor peptide which comprises an amino acid sequence selected from the group consisting of: YLPZ.sub.3 (SEQ ID NO:64), wherein Z.sub.3 is selected from the group consisting of Q, H, N, and W; and GYDMPHVL (SEQ ID NO:4); wherein the tyrosine is phosphorylated; and determining whether the binding of the STAT4 polypeptide to the receptor peptide is increased or decreased in comparison to an assay which lacks the potential binding modulator.

2. The method of claim 1, wherein the receptor peptide comprises an amino acid sequence selected from the group consisting of YLPZ.sub.3 N (SEQ ID NO:14) and GYLPZ.sub.3 (SEQ ID NO:15).

3. The method of claim 2, wherein the peptide comprises an amino acid sequence GYLPZ.sub.3 NID (SEQ ID NO:3).

4. The method of claim 3, wherein the amino acid sequence is selected from the group group consisting of SHEGYLPQNID (SEQ ID NO:18), SHEGYLPHNID (SEQ ID NO:19), SHEGYLPNNID (SEQ ID NO:20), and SHEGYLPWNID) (SEQ ID NO:21).

5. The method of claim 1, wherein the receptor peptide is immobilized on a solid support.

6. The method of claim 5, wherein the binding of the STAT4 polypeptide to the receptor peptide is detected by contacting the STAT4 polypeptide with a detection moiety which binds to the STAT4 polypeptide and comprises a detectable label.

7. The method of claim 6, wherein the STAT4 polypeptide is preincubated with the detection moiety prior to incubating the receptor peptide with the STAT4

polypeptide.

8. The method of claim 5, wherein the binding of the STAT4 polypeptide to the receptor peptide is detected by contacting the STAT4 polypeptide with a primary antibody which binds to the STAT4 polypeptide, and contacting the primary antibody with a detection moiety which binds to the primary antibody and comprises a detectable label.

9. The method of claim 8, wherein the primary antibody is preincubated with the detection moiety prior to incubating the primary antibody with the STAT4 polypeptide.

10. The method of claim 1, wherein the STAT4 polypeptide is immobilized on a solid support.

11. The method of claim 10, wherein the STAT4 polypeptide is immobilized by noncovalent interaction with the solid support.

12. The method of claim 11, wherein the STAT4 polypeptide comprises a polyhistidine sequence and the solid support comprises Ni.sup.2+.

13. The method of claim 11, wherein the STAT4 polypeptide comprises a hydrophobic surface and the STAT4 polypeptide is immobilized by hydrophobic interaction.

14. The method of claim 10, wherein the STAT4 polypeptide is immobilized by covalent covalent attachment to the solid support.

15. The method of claim 10, wherein the receptor peptide is detected by contacting the receptor peptide with a detection moiety which binds to the receptor peptide and comprises a detectable label.

16. The method of claim 15, wherein the receptor peptide is preincubated with the detection moiety prior to incubating the receptor peptide with the STAT4 polypeptide.

17. The method of claim 15, wherein the receptor peptide comprises a tag to which the detection moiety binds.

18. The method of claim 15, wherein the detection moiety comprises an antibody which which binds to the receptor peptide.

19. The method wherein the detection moiety binds indirectly to the receptor peptide.

20. The method of claim 19, wherein the receptor peptide is contacted with a primary primary antibody which binds to the receptor peptide, and the primary antibody is contacted with a secondary antibody which comprises the detection moiety.

21. The method of claim 20, wherein the primary antibody is preincubated with the detection moiety prior to contacting the primary antibody with the receptor peptide.

☐ 3. Document ID: US 6667179 B1

L22: Entry 3 of 13 File: USPT Dec 23, 2003

DOCUMENT-IDENTIFIER: US 6667179 B1
TITLE: Semiconductor luminescence quenchers for detecting proximal molecular binding binding events

CLAIMS:

1. A method for detecting binding or unbinding of first and second molecules, said method comprising the steps of (a) forming a mixture comprising a first molecule, a second molecule and a substrate, wherein: the first molecule comprises a luminophore, one of the first and second molecules is immobilized on the substrate, and the substrate comprises a semiconductor which acts as a luminescence quencher to provide distance-dependent quenching of the luminophore, and between the semiconductor and the immobilized molecule, a molecular attachment layer, whereby but for an incubation-induced change in binding of the first and second molecules, the luminophore and substrate are at a reference distance which provides a reference quenching, whereby the mixture provides a reference luminescence, (b) incubating the mixture under conditions wherein the binding of the first and second molecules changes, whereby the luminophore and substrate are at a test distance which provides a test quenching, whereby the mixture provides a test luminescence, (c) detecting the test luminescence, wherein a difference between the test luminescence and the reference luminescence indicates binding or unbinding of the first and second molecules, wherein the semiconductor has a band gap of less than or equal to about 2 eV and is selected from silicon and germanium, and the substrate is a wafer of the semiconductor.

2. The method of claim 1: wherein the forming step, the second molecule is immobilized on the substrate and the first molecule is bound to the second molecule, molecule, and wherein the incubating step the first and the second molecule unbind, releasing the luminophore from the substrate.

3. The method of claim 2, wherein the semiconductor is silicon.

4. The method of claim 2, wherein the luminophore comprises a lanthanide dye.

5. The method of claim 2, wherein the molecular attachment layer comprises a glass, the semiconductor is silicon and the luminophore comprises a lanthanide dye.

6. The method of claim 1: wherein the forming step, the second molecule is immobilized on the substrate and the first molecule is bound to the second molecule, molecule, and wherein the incubating step the first and the second molecule unbind, releasing the luminophore from the substrate, wherein the first molecule is selected from the group consisting of an antigen, cytokine, hormone and a neurotransmitter and the second molecule is a corresponding receptor.

7. The method of claim 1: wherein the forming step, the second molecule is immobilized on the substrate and the first molecule is bound to the second molecule, molecule, and wherein the incubating step the first and the second molecule unbind, releasing the luminophore from the substrate, wherein the first molecule is selected from the group consisting of an antigen, cytokine, hormone and a neurotransmitter and the second molecule is a corresponding receptor, wherein the forming step, the mixture further comprises a modulator which modulates binding of the first and second molecules.

8. The method of claim 1: wherein the forming step, the first molecule is immobilized on the substrate and the second molecule is unbound to the first molecule, and wherein the incubating step the first and the second molecule bind and release the luminophore from the substrate.

9. The method of claim 8, wherein the semiconductor is silicon.

10. The method of claim 8, wherein the luminophore comprises a lanthanide dye.

11. The method of claim 8, wherein the molecular attachment layer comprises a glass, glass, the semiconductor is silicon and the luminophore comprises a lanthanide dye.

12. The method of claim 1: wherein the forming step, the first molecule is immobilized on the substrate and the second molecule is unbound to the first molecule, and wherein the incubating step the first and the second molecule bind and release the luminophore from the substrate, wherein the second molecule is an enzyme selected from the group consisting of a protease, nuclease, helicase, kinase and phosphatase.

13. The method of claim 1 wherein the molecular attachment layer comprises a glass.

14. The method of claim 13, wherein the semiconductor is silicon.

15. The method of claim 13, wherein the luminophore comprises a lanthanide dye.

16. The method of claim 1 wherein the substrate further comprises a phospholipid bilayer between the molecular attachment layer and the immobilized molecule.

17. The method of claim 16, wherein the semiconductor is silicon.

18. The method of claim 16, wherein the luminophore comprises a lanthanide dye.

19. The method of claim 1 wherein the substrate further comprises a phospholipid bilayer between the molecular attachment layer and the immobilized molecule, and wherein the forming step, the immobilized molecule is bound to or in the bilayer.

20. The method of claim 1, wherein the semiconductor is silicon.

21. The method of claim 1, wherein the luminophore comprises a lanthanide dye.

☐ 4. Document ID: US 6548266 B1

L22: Entry 4 of 13 File: USPT Apr 15, 2003

DOCUMENT-IDENTIFIER: US 6548266 B1
TITLE: Assay for detecting the enzymatic activity of a phosphorylation enzyme using enhanced signal generation

CLAIMS:

1. An improved method for screening samples to perform a phosphorylation assay, wherein the sensitivity of said assay is improved relative to an unconjugated peptide substrate by the method comprising: conjugating a peptide substrate to a signal enhancing polymer; immobilizing said polymer and conjugated peptide substrate substrate on a solid surface; exposing said immobilized substrate to a mixture comprising a candidate modulating agent for phosphorylation; a phosphorylation

enzyme, and detectably labeled source of phosphate groups, for a period of time sufficient for said enzyme to act on said substrate, and measuring the degree of phosphorylation of the substrate.

2. The method of claim 1 wherein said phosphorylation enzyme is a phosphatase.

3. The method of claim 1 wherein said phosphorylation enzyme is a protein kinase.

4. The method of claim 3, wherein said protein kinase is a serine/threonine kinase.

5. The method of claim 1, wherein said peptide substrates are conjugated to a polymer and immobilized on a solid surface in a single step.

6. The method of claim 1, wherein said peptide substrates are conjugated to said signal enhancing polymer via a bifunctional linker.

7. The method of claim 1, wherein said candidate modulating agent for phosphorylation is an inhibitor of phosphorylation.

8. The method of claim 1 wherein said candidate modulating agent for phosphorylation phosphorylation is an enhancer of phosphorylation.

9. The method of claim 1, wherein the polymer is a polyamine.

10. The method of claim 9, wherein the polyamine is polylysine.

11. The method of claim 10, wherein said polylysine polymer is from 400 to 500,000 Daltons in weight.

12. The method of claim 10, wherein said polylysine polymer is from 30,000 to 250,000 Daltons in weight.

13. The method of claim 1, wherein said solid surface comprises a multiwell plate.

14. The method of claim 1, wherein the solid surface is a microsphere.

15. The method of claim 6, wherein said bifunctional linker comprises a succinimidyl succinimidyl ester and a maleimide or iodoacetamide.

16. An improved method for affinity purification of phosphorylation enzymes, wherein wherein the sensitivity is improved by the method comprising: conjugating a peptide substrate to a signal enhancing polymer; immobilizing said polymer and conjugated peptide substrate on a matrix; exposing said immobilized substrate to a complex mixture comprising a candidate phosphorylation enzyme and detectably labeled source of phosphate groups; for a period of time sufficient for said enzyme to bind to said substrate; washing said matrix free of unbound material; and eluting said candidate phosphorylation enzyme from said matrix.

17. The method of claim 16 wherein said phosphorylation enzyme is a phosphatase.

18. The method of claim 16 wherein said phosphorylation enzyme is a protein kinase.

19. The method of claim 18, wherein said protein kinase is a serine/threonine kinase.

20. The method of claim 16, wherein said peptide substrates are conjugated to said signal enhancing polymer via a bifunctional linker.

21. The method of claim 16, wherein the polymer is a polyamine.

22. The method of claim 21, wherein the polyamine is polylysine.

23. The method of claim 21, wherein said polylysine polymer is from 400 to 500,000 Daltons in weight.

24. The method of claim 21, wherein said polylysine polymer is from 30,000 to 250,000 Daltons in weight.

25. The method of claim 20, wherein said bifunctional linker comprises a succinimidyl ester and a maleimide or iodoacetamide.

☐ 5. Document ID: US 6514689 B2

L22: Entry 5 of 13 File: USPT Feb 4, 2003

DOCUMENT-IDENTIFIER: US 6514689 B2
TITLE: Hydrogel biosensor

CLAIMS:

1. A biosensor for measuring the concentration of molecules of an analyte in a body fluid, comprising: a polymeric hydrogel having pendant moieties that are charged under physiological conditions; an analyte binding molecule immobilized in the hydrogel and capable of binding the free analyte; analyte molecules immobilized in the hydrogel; and pressure detection means for measuring the osmotic pressure of the hydrogel.

8. The biosensor of claim 1 wherein the analyte binding molecule is selected from the group consisting of: antibodies, enzymes, membrane receptors, kinases, Protein A, Poly U, Poly A, Poly lysine, triazine dye, nucleoside, boronic acid, thiol, heparin, polysaccharides, Coomassie blue, azure A, and metal-binding peptides, proteins, and chelating agents.

9. The biosensor of claim 1 wherein the immobilized analyte is selected from the group consisting of: antigens, enzyme cofactors, enzyme substrates, enzyme inhibitors, IGG, sugar, carbohydrate, nucleic acids, nucleotide, nucleoside, cysteine, arginine, lysine, protamine, heparin, dyes, and metal ions.

11. The biosensor of claim 1, wherein the immobilized analyte molecules and immobilized analyte binding molecule are present at respective densities chosen to optimize the amount of hydrogel swelling in response to changes in level of free analyte molecules.

17. A method of determining the concentration of free analyte in a solution, comprising the steps of: providing a hydrogel having pendant charged moieties, analyte molecules, and analyte-specific binding molecules covalently immobilized therein; enclosing the hydrogel in a rigid structure which has at least one permeable portion available for contacting a test fluid with the hydrogel, the permeable portion constructed to permit free analyte to diffuse into the hydrogel; contacting the hydrogel sequentially with a series of calibration solutions having known concentrations of free analyte; measuring osmotic pressure in the hydrogel

for each of the calibration solutions to produce a calibration curve of osmotic pressure versus analyte concentration; contacting the hydrogel with the test fluid, and measuring a resulting osmotic pressure; and comparing the resulting osmotic pressure with the calibration curve to determine analyte concentration of the test fluid.

18. The method of claim 17, wherein said steps of measuring the osmotic pressure are are accomplished by disposing pressure sensing means within the rigid structure and in contact with the hydrogel for measuring osmotic pressure of the hydrogel and producing a data signal reflective thereof.

19. The method of claim 18, wherein said pressure sensing means comprises a diaphragm disposed within the rigid enclosure in contact with the hydrogel, and a pressure transducer operably engaged with the diaphragm to measure pressure on the diaphragm.

20. A sensor for measuring the concentration of free molecules of an analyte in a fluid, comprising: a rigid enclosure having an open end and a closed end, the open end being covered by a semipermeable membrane; a diaphragm positioned within the enclosure between the semipermeable membrane and the closed end; a polymeric hydrogel having pendant moieties which are charged at physiological pH, the hydrogel being enclosed between the semipermeable membrane and the diaphragm such that changes in osmotic pressure within the hydrogel are accompanied by changes in pressure exerted on the diaphragm; analyte binding molecules immobilized within the hydrogel; analyte molecules immobilized within the hydrogel; and a pressure transducer operatively engaged to the diaphragm.

22. A method for using a biosensor to measure the concentration of free molecules of of an analyte in a fluid, including: a first step of providing a biosensor comprising: a rigid, biocompatible enclosure having an open end and a closed end, the open end being covered by a semipermeable membrane; a diaphragm positioned between the semipermeable membrane and the closed end such that changes in osmotic pressure within the hydrogel are accompanied by changes in pressure exerted on the diaphragm; a polymeric hydrogel having pendant moieties which are charged at physiological pH, the hydrogel being enclosed between the semipermeable membrane and the diaphragm such that changes in osmotic pressure within the hydrogel are accompanied by changes in pressure exerted on the diaphragm; analyte binding molecules immobilized within the hydrogel; analyte immobilized within the hydrogel, and osmotic pressure sensing means operatively engaged to the diaphragm for sensing the osmotic pressure exerted thereon and providing a data signal reflective thereof; a second step of providing computing means connected to receive the data signal, compare it to a predetermined calibration curve of osmotic pressure vs, concentration of free analyte molecules, and output a concentration value; a third step of inserting the biosensor into the fluid and allowing sufficient time for free analyte molecules to diffuse to equilibrium within the hydrogel; and a fourth step of reading the concentration value output by the computing means.

☐ 6. Document ID: US 6251621 B1

L22: Entry 6 of 13 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251621 B1

TITLE: Reporter enzyme release technology: methods of assaying for the presence of aspartic proteases and other hydrolytic enzyme activities

CLAIMS:

1. A method for assaying for the presence of an enzymatically active hydrolase in a sample, said method comprising:

(a) placing said sample in a test device such that said sample contacts a first solid support in said test device, said first solid support having a reporter enzyme in dry form and covalently attached thereto in such a manner whereby said reporter enzyme is released upon action of said hydrolase, and such that said sample simultaneously contacts a second solid support having immobilized thereon in dry form an indicator, said indicator being one which is susceptible to a detectable change upon action of said reporter enzyme but not susceptible to said change in the absence of said sample; and

(b) observing whether said indicator undergoes a detectable change, said detectable change being an indication of the presence of said enzymatically active hydrolase in said sample.

2. A method in accordance with claim 1 wherein said hydrolase is selected from the group consisting of proteases, peptidases, lipases, nucleases, homo-oligosaccharidases, hetero-oligosaccharidases, homo-polysaccharidases, hetero-polysaccharidases, phosphatases, sulfatases, neuraminidases and esterases.

3. A method in accordance with claim 2 wherein said hydrolase is a protease selected selected from the group consisting of aspartic proteases, serine proteases, thiol proteases, metallo proteases, acid proteases and alkaline proteases.

4. A method in accordance with claim 3 wherein said protease is an aspartic protease.

5. A method in accordance with claim 3 wherein said protease is a thiol protease.

6. A method in accordance with claim 3 wherein said protease is a serine protease.

7. A method in accordance with claim 2 wherein said hydrolase is selected from the group consisting of homo-oligosaccharidases, hetero-oligosaccharidases, homo-polysaccharidases and hetero-polysaccharidases.

8. A method in accordance with claim 7 wherein said hydrolase is selected from the group consisting of chitinase, cellulase, amylase and lysozyme.

9. A method in accordance with claim 1 wherein said reporter enzyme is a signal generating enzyme not subject to inactivation by any agent in said sample, including inactivating hydrolysis by any hydrolase activity present in said sample.

10. A method in accordance with claim 9 wherein said reporter enzyme is selected from the group consisting of peroxidases, phosphatases, oxidoreductases, dehydrogenases, transferases, isomerases, kinases, reductases, deaminases, catalases, urease and glucuronidase.

11. A method in accordance with claim 10 wherein said reporter enzyme is a peroxidase.

12. A method in accordance with claim 11 wherein said peroxidase is horseradish peroxidase.

13. A method in accordance with claim 1 wherein said first and second solid supports supports are independently selected from the group consisting of cellulose, agarose, dextran, polyacrylate, and polyacrylamide.

14. A method in accordance with claim 1 wherein said first and second solid supports supports are independently selected from the group consisting of chitin, sepharose, oxirane acrylic beads, polymeric dialdehyde, starch, collagen, keratin, elastin, bovine hide powder, bacterial cell wall peptidoglycan, nylon, polyethylene terephthalates, polycarbonates and controlled pore glass.

15. A method in accordance with claim 1 wherein said reporter enzyme is colavently attached to said first solid support through a linker molecule which is a substrate for said hydrolase.

16. A method in accordance with claim 15 wherein said linker molecule is selected from the group consisting of proteins, carbohydrates, lipids, peptides, esters and nucleic acids.

17. A method in accordance with claim 16 wherein said linker molecule is a protein selected from the group consisting of azocasein, casein, .kappa.-casein, immunoglobulins, hemoglobin, myoglobin, albumin, elastin, keratin and collagen.

18. A method in accordance with claim 1 wherein said indicator is a visual indicator.

19. A method in accordance with claim 18 wherein said visual indicator is a chromogenic indicator.

20. A method in accordance with claim 19 wherein said chromogenic indicator is an indicator for peroxidative activity.

21. A method in accordance with claim 20 wherein said chromogenic indicator is comprised of a hydroperoxide and a chromogen selected from the group consisting of guaiac, 2-2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid), tetramethylbenzidine, phenol, 4-aminoantipyrine, and 4,5-dihydroxynaphthalene-2,7-disulfonic acid.

22. A method in accordance with claim 1 wherein an inhibitor of a hydrolase other than said hydrolase being assayed for is added to increase specificity for said hydrolase.

23. A method for assaying for the presence of an enzymatically active hydrolase in a a sample, said method comprising:

(a) placing said sample in a device which contains first and second solid supports, said first solid support having a reporter enzyme covalently attached thereto in such a manner whereby said reporter enzyme is released upon action of said hydrolase, said second solid support, which is not in contact with said first solid support, having immobilized thereon an indicator, said indicator being one which is susceptible to a detectable change upon action of said reporter enzyme, said sample being placed in said device in such a manner that said sample contacts said first and second solid supports such that any reporter enzyme released by any hydrolase activity present in said sample is permitted to diffuse through said sample to said second solid support; and

(b) observing whether said indicator undergoes a detectable change, said detectable change being an indication of the presence of said enzymatically active hydrolase in said sample.

24. A method in accordance with claim 23 wherein said hydrolase is selected from the the group consisting of proteases, peptidases, lipases, nucleases, homo-oligosaccharidases, hetero-oligosaccharidases, homo-polysaccharidases, hetero-polysaccharidases, phosphatases, sulfatases, neuraminidases and esterases.

25. A method in accordance with claim 24 wherein said hydrolase is a protease selected from the group consisting of aspartic proteases, serine proteases, thiol proteases, metallo proteases, acid proteases and alkaline proteases.

26. A method in accordance with claim 25 wherein said protease is an aspartic protease.

27. A method in accordance with claim 24 wherein said hydrolase is selected from the the group consisting of homo-oligosaccharidases, hetero-oligosaccharidases, homo-polysaccharidases, and hetero-polysaccharidases.

28. A method in accordance with claim 27 wherein said hydrolase is selected from the the group consisting of chitinase, cellulase, amylase and lysozyme.

29. A method in accordance with claim 23 wherein said reporter enzyme is a signal generating enzyme not subject to inactivation by any agent in said sample, including inactivating hydrolysis by any hydrolase activity present in said sample.

30. A method in accordance with claim 29 wherein said reporter enzyme is selected from the group consisting of peroxidases, phosphatases, oxidoreductases, dehydrogenases, transferases, isomerases, kinases, reductases, deaminases, catalases, urease and glucuronidase.

31. A method in accordance with claim 30 wherein said reporter enzyme is a peroxidase.

32. A method in accordance with claim 31 wherein said peroxidase is horseradish peroxidase.

33. A method in accordance with claim 23 wherein said first and second solid supports are independently selected from the group consisting of cellulose, agarose, dextran, polyacrylate, and polyacrylamide.

34. A method in accordance with claim 23 wherein said first and second solid supports are independently selected from the group consisting of chitin, polymeric dialdehyde, starch, nylon, polyethylene terephthalates, polycarbonates and controlled pore glass.

35. A method in accordance with claim 23 wherein said reporter enzyme is covalently attached to said first solid support through a linker molecule which is a substrate for said hydrolase.

36. A method in accordance with claim 35 wherein said linker molecule is selected from the group consisting of proteins, carbohydrates, lipids, peptides, esters and nucleic acids.

37. A method in accordance with claim 36 wherein said linker molecule is a protein selected from the group consisting of azocasein, casein, .kappa.-casein, immunoglobulins, hemoglobin, myoglobin, albumin, elastin, keratin and collagen.

38. A method in accordance with claim 23 wherein said indicator is a visual indicator.

39. A method in accordance with claim 38 wherein said visual indicator is a

chromogenic indicator.

40. A method in accordance with claim 39 wherein said chromogenic indicator is an indicator for peroxidative activity.

41. A method in accordance with claim 40 wherein said chromogenic indicator is comprised of a hydroperoxide and a chromogen selected from the group consisting of guaiac, 2-2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid), tetramethylbenzidine, phenol, 4-aminoantipyrine, and 4,5-dihydroxynaphthalene-2,7-disulfonic acid.

42. A method in accordance with claim 41 wherein said chromogenic indicator is comprised of a hydroperoxide and guaiac.

43. A method in accordance with claim 23 wherein an inhibitor of a hydrolase other than said hydrolase being assayed for is added to increase specificity for said hydrolase.

44. A method for assaying for the presence of enzymatically active aspartic protease protease in a sample, said method comprising:

(a) placing said sample in a device which contains first and second solid supports, said first solid support being polyacrylate and having horseradish peroxidase covalently attached thereto through myoglobin which is a substrate for said aspartic protease, said second solid support, which is not in contact with said first solid support, being hydroxypropyl cellulose and having immobilized thereon a hydroperoxide and guaiac, a chromogenic substrate which undergoes a color change upon action of said horseradish peroxidase in the presence of said hydroperoxide, said sample being placed in said device in such a manner that said sample contacts said first and second solid supports such that any horseradish peroxidase released by any aspartic protease present in said sample is permitted to diffuse through said sample to said second solid support; and

(b) observing whether said guaiac undergoes a color change, said color change being an indication of the presence of said enzymatically active aspartic protease in said sample.

45. A method for detecting candidiasis by assaying for the presence of enzymatically enzymatically active aspartic protease in a sample, said method comprising:

(a) contacting said sample with a first solid support, said first solid support having a reporter enzyme covalently attached thereto in such a manner whereby said reporter enzyme is released upon action of said aspartic protease;

(b) combining said sample with a second solid support, said second solid support having immobilized thereon an indicator, said indicator being one which is susceptible to a detectable change upon action of said reporter enzyme; and

(c) observing whether said indicator undergoes a detectable change, said detectable change being an indication of the presence of said enzymatically active aspartic protease in said sample and thus, candidiasis.

46. A method in accordance with claim 45 wherein said reporter enzyme is a signal generating enzyme not subject to inactivation by any agent in said sample, including inactivating hydrolysis by any hydrolase activity present in said sample.

47. A method in accordance with claim 45 wherein said first and second solid supports are independently selected from the group consisting of cellulose, agarose, dextran, polyacrylate, and polyacrylamide.

48. A method in accordance with claim 45 wherein said solid support is selected from from the group consisting of chitin, sepharose, oxirane acrylic beads, polymeric dialdehyde, starch, collagen, keratin, elastin, bovine hide powder, bacterial cell wall peptidoglycan, nylon, polyethylene terephthalates, polycarbonates and controlled pore glass.

49. A method in accordance with claim 45 wherein said reporter enzyme is covalently attached to said first solid support through a linker molecule which is a substrate for said hydrolase.

50. A method in accordance with claim 49 wherein said linker molecule is selected from the group consisting of proteins and peptides.

51. A method in accordance with claim 50 wherein said protein is selected from the group consisting of azocasein, casein, .kappa.-casein, immunoglobulins, hemoglobin, myoglobin, albumin, elastin, keratin and collagen.

52. A method in accordance with claim 45 wherein said indicator is a visual indicator.

53. A method in accordance with claim 54 wherein said visual indicator is a chromogenic indicator.

54. A method in accordance with claim 45 wherein the pH of said sample is from about about 2.5 to about 5.0 to increase aspartic protease sensitivity and specificity.

55. A method in accordance with claim 45 wherein a protease inhibitor is added to increase specificity for aspartic protease activity.

56. A method in accordance with claim 55 wherein said protease inhibitor is selected selected from the group consisting of inhibitors of serine proteases, thiol proteases, metallo proteases and other non-aspartic proteases.

57. A method for detecting Trichomonas vaginalis by assaying for the presence of enzymatically active thiol protease in a sample, said method comprising:

(a) contacting said sample with a first solid support, said first solid support having a reporter enzyme covalently attached thereto in such a manner whereby said reporter enzyme is released upon action of said thiol protease;

(b) combining said sample with a second solid support having immobilized thereon an indicator, said indicator being one which is susceptible to a detectable change upon action of said reporter enzyme; and

(c) observing whether said indicator undergoes a detectable change, said detectable change being an indication of the presence of said enzymatically active thiol protease in said sample and thus, Trichomonas vaginalis.

58. A method in accordance with claim 57 wherein said reporter enzyme is a signal generating enzyme not subject to inactivation by any agent in said sample, including inactivating hydrolysis by any hydrolase activity present in said sample.

59. A method in accordance with claim 57 wherein said first and second solid supports are independently selected from the group consisting of cellulose, agarose, dextran, polyacrylate, and polyacrylamide.

60. A method in accordance with claim 57 wherein said first and second solid supports are independently selected from the group consisting of chitin, sepharose,

oxirane acrylic beads, polymeric dialdehyde, starch, collagen, keratin, elastin, bovine hide powder, bacterial cell wall peptidoglycan, nylon, polyethylene terephthalates, polycarbonates and controlled pore glass.

61. A method in accordance with claim 57 wherein said reporter enzyme is covalently attached to said first solid support through a linker molecule which is a substrate for said hydrolase.

62. A method in accordance with claim 61 wherein said linker molecule is selected from the group consisting of proteins and peptides.

63. A method in accordance with claim 62 wherein said protein is selected from the group consisting of azocasein, casein, .kappa.-casein, immunoglobulins, hemoglobin, myoglobin, albumin, elastin, keratin and collagen.

64. A method in accordance with claim 57 wherein said indicator is a visual indicator.

65. A method in accordance with claim 64 wherein said visual indicator is a chromogenic indicator.

66. A method for assaying for the presence of an inhibitor of a target hydrolase in a sample, said method comprising:

(a) contacting said sample with said target hydrolase and a first solid support, said first solid support having a reporter enzyme covalently attached thereto in such a manner whereby said reporter enzyme is released upon action of said target hydrolase if said target hydrolase is not inactivated by the presence of said inhibitor;

(b) combining said sample with a second solid support, said second solid support having immobilized thereon an indicator, said indicator being one which is susceptible to a detectable change upon action of said reporter enzyme; and

(c) observing whether said indicator undergoes a detectable change, said detectable change being an indication of the absence of said inhibitor of said target hydrolase in said sample.

67. A method in accordance with claim 66 wherein said target hydrolase is selected from the group consisting of proteases, peptidases, lipases, nucleases, homo-oligosaccharidases, hetero-oligosaccharidases, homo-polysaccharidases, hetero-polysaccharidases, phosphatases, sulfatases, neuraminidases and esterases.

68. A method in accordance with claim 67 wherein said target hydrolase is a protease protease selected from the group consisting of aspartic proteases, serine proteases, thiol proteases, metallo proteases, acid proteases and alkaline proteases.

69. A method in accordance with claim 68 wherein said protease is an aspartic protease.

70. A method in accordance with claim 66 wherein said inhibitor is selected from the the group consisting of inhibitors of proteases, peptidases, lipases, nucleases, homo-oligosaccharidases, hetero-oligosaccharidases, homo-polysaccharidases, hetero-polysaccharidases, phosphatases, sulfatases, neuraminidases and esterases.

71. A method in accordance with claim 70 wherein said inhibitor is an inhibitor of proteases.

72. A method in accordance with claim 71 wherein said inhibitor is an inhibitor of

aspartic proteases selected from the group consisting of pepstatin, ovomacroglobulin, haloperidol, transition state mimetics, U-81749, H-261, MV7-101, A-75925, A-76928 and A-7003.

73. A method in accordance with claim 66 wherein said reporter enzyme is a signal generating enzyme not subject to inactivation by any agent present in said sample, including inactivating hydrolysis by any hydrolase activity present in said sample.

74. A method in accordance with claim 73 wherein said reporter enzyme is selected from the group consisting of peroxidases, phosphatases, oxidoreductases, dehydrogenases, transferases, isomerases, kinases, reductases, deaminases, catalases, urease and glucuronidase.

75. A method in accordance with claim 74 wherein said reporter enzyme is a peroxidase.

76. A method in accordance with claim 75 wherein said peroxidase is horseradish peroxidase.

77. A method in accordance with claim 66 wherein said first and second solid supports are independently selected from the group consisting of cellulose, agarose, dextran, polyacrylate, and polyacrylamide.

78. A method in accordance with claim 66 wherein said first and second solid supports are independently selected from the group consisting of chitin, sepharose, oxirane acrylic beads, polymeric dialdehyde, starch, collagen, keratin, elastin, bovine hide powder, bacterial cell wall peptidoglycan, nylon, polyethylene terephthalates, polycarbonates and controlled pore glass.

79. A method in accordance with claim 66 wherein said reporter enzyme is covalently attached to said first solid support through a linker molecule which is a substrate for said target hydrolase.

80. A method in accordance with claim 79 wherein said linker molecule is selected from the group consisting of proteins, carbohydrates, lipids, peptides, esters and nucleic acids.

81. A method in accordance with claim 80 wherein said linker molecule is a protein selected from the group consisting of azocasein, casein, .kappa.-casein, immunoglobulins, hemoglobin, myoglobin, albumin, elastin, keratin and collagen.

82. A method in accordance with claim 66 wherein said indicator is a visual indicator.

83. A method in accordance with claim 82 wherein said visual indicator is a chromogenic indicator.

84. A method in accordance with claim 83 wherein said chromogenic indicator is an indicator for peroxidative activity.

85. A method in accordance with claim 84 wherein said chromogenic indicator is comprised of a hydroperoxide and a chromogen selected from the group consisting of guaiac, 2-2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid), tetramethylbenzidine, phenol, 4-aminoantipyrine, and 4,5dihydroxynaphthalene-2,7-disulfonic acid.

☐ 7. Document ID: US 6207391 B1

L22: Entry 7 of 13 File: USPT Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207391 B1
TITLE: High-throughput screening assays for modulators of STAT4 and STAT6 activity

CLAIMS:

1. A method of screening for modulators of STAT6 binding to a STAT6 receptor, the method comprising:

incubating a reaction mixture comprising a STAT6 polypeptide, a potential binding modulator, and a receptor peptide which comprises an amino acid sequence YX.sub.1 X.sub.2 X.sub.3 (SEQ ID NO:61), wherein the tyrosine is phosphorylated and:

X.sub.1 is selected from the group consisting of K, V, R, I, M, and a first nonnatural amino acid;

X.sub.2 is selected from the group consisting of P, A and S; and

X.sub.3 is selected from the group consisting of W, Y, F, H, L and an aromatic second nonnatural amino acid;

with the proviso that the peptide does not include the amino acid sequence YKPF (SEQ ID NO:2) or YKAF; and

determining whether the binding of the STAT6 polypeptide to the receptor peptide is increased or decreased in comparison to an assay which lacks the potential binding modulator.

2. The method of claim 1 wherein the receptor peptide comprises an amino acid sequence YX.sub.1 X.sub.2 X.sub.3 X.sub.4 (SEQ ID NO:62), wherein X.sub.4 is selected from the group consisting of D and G.

3. The method of claim 1, wherein X.sub.1 is a nonnatural amino acid selected from the group consisting of tert-butyl glycine, norvaline, cyclohexylalanine, and allothreonine.

4. The method of claim 1, wherein X.sub.3 is an aromatic nonnatural amino acid selected from the group consisting of p-iodophenylalanine, 1-napthylalanine, benzothiophenylalanine, 3-iodotyrosine, p-chlorophenylalanine, m-trifluoromethylphenylalanine, and o-chlorophenylalanine.

5. The method of claim 1, wherein the receptor peptide comprises an amino acid sequence selected from the group consisting of EGYVPWQDLI (SEQ ID NO:17), EGYKPZ.sub.1 QDLI (SEQ ID NO:39), and EGYZ.sub.2 PQWDLI (SEQ ID NO:32), wherein:

Z.sub.1 is an aromatic nonnatural amino acid selected from the group consisting of p-iodophenylalanine, 1-napthylalanine, benzothiophenylalanine, 3-iodotyrosine, p-chlorophenylalanine, m-trifluoromethylphenylalanine, and o-chlorophenylalanine; and

Z.sub.2 is a nonnatural amino acid is selected from the group consisting of tert-butyl glycine, norvaline, cyclohexylalanine, and allothreonine.

6. The method of claim 1, wherein the receptor peptide is immobilized on a solid support.

7. A The method of claim 6, wherein the binding of the STAT6 polypeptide to the receptor peptide is detected by contacting the STAT6 polypeptide with a detection moiety which binds to the STAT6 polypeptide and comprises a detectable label.

8. The method of claim 7, wherein the STAT6 polypeptide is preincubated with the detection moiety prior to incubating the receptor peptide with the STAT6 polypeptide.

9. The method of claim 6, wherein the binding of the STAT6 polypeptide to the receptor peptide is detected by contacting the STAT6 polypeptide with a primary antibody which binds to the STAT6 polypeptide, and contacting the primary antibody with a detection moiety which binds to the primary antibody and comprises a detectable label.

10. The method of claim 9, wherein the primary antibody is preincubated with the detection moiety prior to incubating the primary antibody with the STAT6 polypeptide.

11. The method of claim 1, wherein the STAT6 polypeptide is immobilized on a solid support.

12. The method of claim 11, wherein the STAT6 polypeptide is immobilized by noncovalent interaction with the solid support.

13. The method of claim 12, wherein the STAT6 polypeptide comprises a polyhistidine sequence and the solid support comprises Ni.sup.2+.

14. The method of claim 12, wherein the solid support comprises a hydrophobic surface and the STAT6 polypeptide is immobilized by hydrophobic interaction.

15. The method of claim 11, wherein the STAT6 polypeptide is immobilized by covalent covalent attachment to the solid support.

16. The method of claim 11, wherein the receptor peptide is detected by contacting the receptor peptide with a detection moiety which binds to the receptor peptide and comprises a detectable label.

17. The method of claim 16, wherein the receptor peptide is preincubated with the detection moiety prior to incubating the receptor peptide with the STAT6 polypeptide.

18. The method of claim 16, wherein the receptor peptide comprises a tag to which the detection moiety binds.

19. The method of claim 16, wherein the detection moiety comprises an antibody which which binds to the receptor peptide.

20. The method of claim 16, wherein the detection moiety binds indirectly to the receptor peptide.

21. The method of claim 20, wherein the receptor peptide is contacted with a primary primary antibody which binds to the receptor peptide, and the primary antibody is contacted with a secondary antibody which comprises the detection moiety.

22. The method of claim 21, wherein the primary antibody is preincubated with the detection moiety prior to contacting the primary antibody with the receptor

peptide.

☐ 8. Document ID: US 6074852 A

L22: Entry 8 of 13 File: USPT Jun 13, 2000

DOCUMENT-IDENTIFIER: US 6074852 A
TITLE: Hepatitis C virus asialoglycoproteins

CLAIMS:

1. A method for producing an hepatitis C virus (HCV) qlycoprotein having mannose-terminated glycosylation, wherein less than about 10% of the total N-linked carbohydrate on said HCV glycoprotein is sialic acid, and further wherein said HCV qlycoprotein is selected from the group consisting of a glycoprotein expressed from the E1 region of HCV and a glycoprotein expressed from the E2 region of HCV comprising the steps of:

growing a mammalian host cell transformed with a structural gene encoding an HCV glycoprotein expressed from either the E1 region of HCV or the E2 region of HCV in a suitable culture medium;

causing expression of said structural gene under conditions inhibiting sialylation; and

isolating said HCV qlycoprotein from cell culture by contacting said HCV glycoprotein with a mannose-binding protein specific for mannose-terminated glycoproteins.

2. The method of claim 1, wherein said conditions inhibiting sialylation comprise expression of the qlycoprotein at a rate sufficient to inhibit transport of glycoproteins from the endoplasmic reticulum to the golgi.

3. The method according to claim 1, wherein said conditions inhibiting sialylation comprise a sufficient amount of a calcium modulator to cause release of proteins within the host cell's endoplasmic reticulum.

4. The method according to any of claims 1-3, wherein said qlycoprotein is expressed expressed from the E1 region of HCV.

5. The method according to any of claims 1-3, wherein said glycoprotein is expressed expressed from the E2 region of HCV.

6. The method according to any of claims 1-3, wherein said glycoprotein is an E1/E2 aggregate.

7. The method according to any of claims 1-3, wherein said mannose-binding protein is a lectin selected from the group consisting of ConA and GNA.

8. The method according to any of claims 1-3, wherein said mannose-binding protein is immobilized on a support.

9. The method according to claim 8, wherein said contacting comprises incubation of

said expression product in a column comprising a mannose-binding lectin immobilized on a support, for a period of at least one hour; and wherein said isolating comprises eluting said glycoprotein with mannose.

10. The method of any one of claims 1-3, wherein said glycoprotein is an E1/E1 aggregate.

11. The method of any one of claims 1-3, wherein said glycoprotein is an E1/E2 aggregate.

☐ 9. Document ID: US 5834216 A

L22: Entry 9 of 13 File: USPT Nov 10, 1998

DOCUMENT-IDENTIFIER: US 5834216 A
TITLE: Screening methods for the identification of inducers and inhibitors of programmed cell death (apoptosis)

CLAIMS:

1. A method of screening candidate inhibitors of programmed cell death, the method comprising the steps of:

(a) preparing duplicate cell cultures;

(b) exposing one of the duplicate cell cultures to a candidate inhibitor;

(c) exposing the duplicate cell cultures to an inducer of programmed cell death;

(d) preparing respective cell lysates from the duplicate cell cultures of step (c);

(e) contacting the lysates of step (d) with ATP wherein the .gamma. phosphate of the ATP has a detectable label; and

(f) measuring the levels of phosphorylated p90 and/or eIF-2.alpha. produced by the lysates;

whereby inhibitors of programmed cell death are identified by their ability to prevent or decrease phosphorylation of eIF-2.alpha. and/or p90 when compared to the level of phosphorylation of eIF-2.alpha. and/or p90 in cells not exposed to the candidate substance.

2. The method of claim 1 wherein the cells are human cells.

3. The method of claim 2 wherein the human cells are selected from the group consisting of HeLa cells, SK-N-SH neuroblastoma cells, and human foreskin fibroblasts.

4. The method of claim 1 wherein the programmed cell death is induced by infection with a herpes simplex virus lacking .gamma..sub.1 34.5 genes capable of expressing an active gene product.

5. The method of claim 1 wherein the ATP is .gamma..sup.32 P-ATP.

6. The method of claim 1 wherein the levels of phosphorylated eIF-2.alpha. and/or p90 are measured by the steps of:

precipitating eIF-2.alpha. and/or p90 from the cell lysates using an antibody directed to PKR;

separating precipitated eIF-2.alpha. and/or p90 by electrophoresis on denaturing gels;

transferring the separated eIF-2.alpha. and/or p90 onto an immobilizing membrane;

exposing the membrane to X-ray film;

developing the X-ray; and

measuring the intensities of the eIF-2.alpha. specific signal and the p90 specific signal generated from the duplicate cell cultures.

7. A method for screening candidate inducers of programmed cell death the method comprising the steps of:

(a) preparing duplicate cell cultures;

(b) exposing one of the duplicate cell cultures to a candidate inducer of programmed cell death;

(c) preparing respective cell lysates from the cell culture exposed to the candidate inducer and from the unexposed cell culture;

(d) contacting the lysates with ATP wherein the .gamma. phosphate of the ATP has a detectable label; and

(e) measuring the levels of phosphorylated p90 and/or eIF-2.alpha. produced in the lysates;

whereby inducers of programmed cell death are identified by their ability to increase phosphorylation of eIF-2.alpha. and/or p90 in the lysates of exposed cells when compared to lysates from unexposed cells.

8. The method of claim 7 wherein the cells are human cells.

9. The method of claim 8 wherein the cells are selected from the group consisting of of HeLa cells, SK-N-SH neuroblastoma cells, and foreskin fibroblasts.

10. The method of claim 7 wherein the ATP is .gamma..sup.32 P-ATP.

11. The method of claim 7 wherein the levels of phosphorylated eIF-2.alpha. and/or p90 are measured by the steps of:

precitipating eIF-2.alpha. and/or p90 from the cell lysates using an antibody directed to PKR;

separating precipitated PKR-kinase, eIF-2.alpha. and/or p90 by electrophoresis on denaturing gels;

transferring the separated PKR-kinase, eIF-2.alpha. and/or p90 onto an immobilizing membrane;

exposing membrane to X-ray film:

developing the X-ray film; and

measuring the intensities of the PKR-kinase, eIF-2.alpha. specific signal and/or p90 p90 specific signal generated in the duplicate cell cultures.

12. A method of screening candidate inhibitors of programmed cell death, the method comprising the steps of:

(a) preparing duplicate cell cultures;

(b) exposing one of the duplicate cell cultures to a candidate inhibitor;

(c) exposing the duplicate cultures to an inducer of programmed cell death; and,

(d) measuring the levels of phosphorylated p90 and/or eIF-2.alpha. in each of the duplicate cell cultures;

whereby inhibitors of programmed cell death are identified by their ability to prevent or decrease the level of phosphorylation of eIF-2.alpha. and/or p90 when compared to the level of phosphorylation of eIF-2.alpha. and/or p90 in cells not exposed to the candidate inhibitor.

13. The method of claim 12 further comprising the steps of:

(e) exposing duplicate cell cultures of step (c) to [.sup.35 S]-methionine;

(f) preparing a lysate of the duplicate cell cultures of step (e);

(g) separating the phosphorylated and unphoshorylated eIF-2.alpha. and/or p90 contained in the lysate from other constituents of the lysate; and

(h) measuring the level of .sup.35 S-labelled phosphorylated and unphosphorylated eIF-2.alpha. and/or p90;

whereby an inhibitor of programmed cell death is identified by its ability to decrease or inhibit the phosphorylation of eIF-2.alpha. and/or p90.

14. The method of claim 13 wherein said phosphorylated and unphosphorylated eIF-2.alpha. and/or p90 are separated from other constituents of the lysate by polyacrylamide gel electrophoresis.

15. A method for screening candidate inducers of programmed cell death, the method comprising the steps of:

(a) preparing duplicate cell cultures;

(b) exposing one of the duplicate cultures to a candidate inducer of programmed cell death; and

(c) measuring the levels of phosphorylated eIF-2.alpha. and/or p90 in each of the duplicate cultures;

wherein an inducer of programmed cell death is identified by its ability to increase the level of phosphorylation of eIF-2.alpha. and/or p90.

16. The method of claim 15 further comprising the steps of:

(d) exposing said duplicate cultures of steps (a) and (b) to [.sup.35 S]-

methionine;

(e) preparing a lysate of each of the duplicate cell cultures of step (d);

(f) separating the phosphorylated and unphoshorylated eIF-2.alpha. and/or p90 contained in the lysates from other constituents of the lysates; and

(g) measuring the relative .sup.35 S-labelled phosphorylated and unphosphorylated eIF-2.alpha. and/or p90;

whereby an inducer of programmed cell death is identified by its ability to increase the level of phosphorylation of eIF-2.alpha. and/or p90.

17. The method of claim 16 wherein said phosphorylated and unphosphorylated eIF-2.alpha. and/or p90 are separated from other constituents of the lysates by polyacrylamide gel electrophoresis.

☐ 10. Document ID: US 5744313 A

L22: Entry 10 of 13 File: USPT Apr 28, 1998

DOCUMENT-IDENTIFIER: US 5744313 A
TITLE: Assay employing novel protein domain which binds tyrosine phosphorylated proteins

CLAIMS:

1. A method of determining whether a test compound is an agonist or antagonist of a growth factor activation signaling pathway in a cell, comprising:

contacting the cell with the test compound in the presence of a growth factor;

lysing the cell to produce a cell lysate; and

determining an amount of a phosphorylated ligand to a PTB domain present in said cell lysate, said determining step comprising the steps of contacting the cell lysate with an isolated polypeptide which comprises a PTB domain, and which isolated polypeptide does not contain an SH2 domain, and determining an amount of protein in the cell lysate that binds to said isolated polypeptide; and

comparing the amount of phosphorylated ligand to a PTB domain from said determining step to an amount of phosphorylated ligand to a PTB domain in a cell lysate prepared prepared from a cell contacted with a growth factor in the absence of said test compound, an increase or decrease in the amount of said phosphorylated ligand in the presence of said test compound being indicative that the compound is an agonist or antagonist of the growth factor activation signaling pathway, respectively.

2. The method of claim 1, wherein in said contacting step, said growth factor is selected from the group consisting of PDGF, FGF, EGF, insulin and insulin-like growth factor.

3. A method of determining if a test compound is an agonist or antagonist of PTB domain/phosphorylated ligand mediated regulatory system, the method comprising:

incubating the test compound with a polypeptide that comprises a PTB domain and which polypeptide does not contain an SH2 domain, and a phosphorylated ligand which is capable of interacting with said PTB domain to form a PTB domain/phosphorylated ligand complex, under conditions which permit the formation of said complex;

determining an amount of PTB domain/phosphorylated ligand complex formed in the presence of said test compound, and comparing said amount to an amount of PTB domain/phosphorylated ligand complex formed in the absence of the test compound;

an increase or decrease in the amount of PTB domain/phosphorylated ligand complex formed in the presence of the test compound over the amount of PTB domain/phosphorylated ligand complex formed in the absence of the test compound being indicative that the test compound is an agonist or antagonist of the PTB domain/phosphorylated ligand mediated regulatory system, respectively.

4. The method of claim 3, wherein said polypeptide comprising a PTB domain is covalently coupled to a solid support, and said phosphorylated ligand which is capable of interacting with said PTB domain is covalently coupled to a detectable group.

5. The method of claim 4, wherein said detectable group is selected from the group consisting of a radiolabel, a fluorescent label, an antibody binding epitope and an assayable enzyme.

6. The method of claim 4, wherein said step of determining the amount of PTB domain/phosphorylated ligand complex formed in the presence and absence of said test test compound comprises determining the amount of detectable group that is bound to said solid support.

7. The method of claim 3, wherein said polypeptide comprising a PTB domain is covalently coupled to a detectable group, and said phosphorylated ligand which is capable of interacting with said PTB domain is covalently coupled to a solid support.

8. The method of claim 7, wherein said detectable group is selected from the group consisting of a radiolabel, a fluorescent label, an antibody binding epitope and an assayable enzyme.

9. The method of claim 7, wherein said step of determining the amount of PTB domain/phosphorylated ligand complex formed in the presence and absence of said test test compound comprises determining the amount of detectable group that is bound to said solid support.

10. The method of claim 3, wherein said PTB domain/phosphorylated ligand mediated regulatory system is a growth factor activation signaling pathway in a cell.

11. The method of claim 10, wherein said growth factor activation signaling pathway is selected from the group consisting of PDGF, FGF, Insulin and Insulin-like growth factor activation signaling pathways.

12. A method of determining whether a test compound is an agonist or antagonist of a a growth factor activation signaling pathway in a cell, comprising:

contacting the cell with the test compound in the presence of a growth factor;

lysing the cell to produce a cell lysate;

determining an amount of a phosphorylated ligand to a PTB domain present in said cell lysate, wherein said determining step comprises the steps of:

contacting the cell lysate with an isolated polypeptide having an amino acid sequence of a SCK phosphotyrosine binding domain, as shown in FIG. 4 (SEQ ID NO:2), or a phosphotyrosine binding fragment thereof; and

determining an amount of protein in the cell lysate that binds to said polypeptide; and

comparing the amount of phosphorylated ligand to a PTB domain from said determining step to an amount of phosphorylated ligand to a PTB domain in a cell lysate prepared prepared from a cell contacted with a growth factor in the absence of said test compound, an increase or decrease in the amount of said phosphorylated ligand in the presence of said test compound being indicative that the compound is an agonist or antagonist of the growth factor activation signaling pathway, respectively.

13. The method of claim 12, wherein said polypeptide is covalently coupled to a detectable group, said detectable group being selected from the group consisting of a radiolabel, a fluorescent label an antibody binding epitope and an assayable enzyme.

14. The method of claim 13, wherein said cell lysate is immobilized upon a solid support, and said determining step comprises assaying for the presence of said detectable group bound to said solid support.

15. The method of claim 14, wherein said solid support is nitrocellulose.

16. The method of claim 14, wherein said solid support is a microtiter plate.

17. The method of claim 12, wherein said polypeptide is immobilized upon a solid support, and said determining step comprises determining an amount of protein from said cell lysate that binds to said solid support.

18. The method of claim 12, wherein said solid support is nitrocellulose.

19. The method of claim 12, wherein said solid support is a microtiter plate.

20. A method of determining if a test compound is an agonist or antagonist of PTB domain/phosphorylated ligand mediated regulatory system, the method comprising:

incubating the test compound with a polypeptide that comprises a PTB domain and which polypeptide does not contain an SH2 domain, and a phosphorylated ligand which is capable of interacting with said PTB domain to form a PTB domain/phosphorylated ligand complex, under conditions which permit the formation of said complex, wherein said polypeptide comprising a PTB domain has an amino acid sequence of a SCK phosphotyrosine binding domain as shown in FIG. 4 (SEQ ID NO:2), or a phosphotyrosine binding fragment thereof; and

determining an amount of PTB domain/phosphorylated ligand complex formed in the presence of said test compound, and comparing said amount to an amount of PTB domain/phosphorylated ligand complex formed in the absence of the test compound;

an increase or decrease in the amount of PTB domain/phosphorylated ligand complex formed in the presence of the test compound over the amount of PTB domain/phosphorylated ligand complex formed in the absence of the test compound being indicative that the test compound is an agonist or antagonist of the PTB domain/phosphorylated ligand mediated regulatory system, respectively.

21. The method of claim 20, wherein in said incubating step, said phosphorylated ligand which is capable of interacting with said PTB domain is pp145.

11. Document ID: US 5565326 A

L22: Entry 11 of 13 File: USPT Oct 15, 1996

DOCUMENT-IDENTIFIER: US 5565326 A
TITLE: Separation-free specific binding assays using anti-inhibitor antibodies

CLAIMS:

1. A separation-free specific binding assay comprising:

A) contacting together, in any order,

1) a fluid sample suspected of containing a target specific binding ligand,

2) an immobilized receptor for said target specific binding ligand,

3) a reporter enzyme,

4) an inhibitor antibody having the following characteristics:

a) specifically binds to said reporter enzyme,

b) a dissociation constant less than or equal to about 125 nmolar, and

c) specifically binds to said reporter enzyme in such a manner as to inhibit the enzymatic activity of said reporter enzyme by at least about 80%, and

5) a water soluble conjugate of said target specific binding ligand and an anti-inhibitor antibody having the following characteristics:

a) specifically binds to said reporter enzyme,

b) a dissociation constant less than or equal to about 50 nmolar, and

c) specifically binds to said reporter enzyme in such a manner that the enzymatic activity of said reporter enzyme is diminished by no more than 20%, and wherein said anti-inhibitor antibody prevents said inhibitor antibody from binding with said said reporter enzyme,

to form a reaction mixture comprising a complex between said immobilized receptor and either said target specific binding ligand or said water-soluble conjugate,

B) contacting said reaction mixture simultaneously or sequentially with a signal-generating reagent comprising a substrate for the reporter enzyme, and

C) detecting any signal generated from said reporter enzyme as a determination of said target specific binding ligand in said fluid sample.

2. The method of claim 1 wherein said reporter enzyme is immobilized on a water-insoluble support.

3. The method of claim 2 wherein said reporter enzyme is immobilized on polymeric

particles and wherein said receptor is immobilized on polymeric particles.

4. The method of claim 1 wherein either or both of said inhibitor antibody and said anti-inhibitor antibody are monoclonal antibodies.

5. The method of claim 1 wherein said reporter enzyme is a peroxidase, alkaline phosphatase, creatine kinase, glucose oxidase, urease, glucose-6-phosphate dehydrogenase or beta-galactosidase.

6. The method of claim 1 wherein said reporter enzyme is horseradish peroxidase.

7. The method of claim 1 wherein:

said inhibitor antibody is monoclonal, has a dissociation constant of less than or equal to about 10 nmolar, and binds to said reporter enzyme in such a manner as to inhibit at least about 95% of the enzymatic activity of said reporter enzyme, and

said anti-inhibitor antibody is monoclonal, has a dissociation constant less than or or equal to about 5 nmolar, and binds to said reporter enzyme in such a manner that the enzymatic activity of said reporter enzyme is diminished by no more than about 6%.

8. The method of claim 1 wherein said substrate is a chromogenic substrate and said detecting comprises measuring colored product.

9. The method of claim 1 wherein said target specific binding ligand is diphenylhydantoin, digoxin or phenobarbital; wherein said immobilized receptor is an antibody which specifically binds thereto; and wherein

said water-soluble conjugate is formed from said anti-inhibitor antibody and said target specific binding ligand.

10. The method of claim 1 wherein said fluid sample, said inhibitor antibody and said water-soluble conjugate are contacted together prior to contact with said immobilized receptor or said reporter enzyme.

11. A test kit useful for a separation-free specific binding assay comprising, in individual packaging:

an immobilized receptor for a target specific binding ligand, and

at least two additional reagents selected from the group consisting of:

a reporter enzyme,

an inhibitor antibody having the following characteristics:

a) specifically binds to said reporter enzyme,

b) a dissociation constant less than or equal to about 125 nmolar, and

c) specifically binds to said reporter enzyme in such a manner as to inhibit the enzymatic activity of said reporter enzyme by at least about 80%, and

a water soluble conjugate of said target specific binding ligand and an anti-inhibitor antibody having the following characteristics:

a) specifically binds to said reporter enzyme,

b) a dissociation constant less than or equal to about 50 nmolar, and

c) specifically binds to said reporter enzyme in such a manner that the enzymatic activity of said reporter enzyme is diminished by no more than 20%, and wherein said said anti-inhibitor antibody prevents said inhibitor antibody from binding with said said reporter enzyme.

12. The test kit of claim 11 wherein said reporter enzyme is immobilized on polymeric particles.

13. The test kit of claim 11 wherein either or both of said inhibitor antibody and said anti-inhibitor antibody are monoclonal antibodies.

14. The test kit of claim 11 wherein said reporter enzyme is horseradish peroxidase,

said inhibitor antibody is monoclonal, has a dissociation constant of less than about 10 nmolar, and binds to said horseradish peroxidase in such a manner as to inhibit at least about 95% of the enzymatic activity of said horseradish peroxidase, and

said anti-inhibitor antibody is monoclonal, has a dissociation constant greater than than or equal to about 5 nmolar, and binds to said horseradish peroxidase in such a manner that the enzymatic activity of said horseradish peroxidase is diminished by no more than about 6%.

15. The kit of claim 11 wherein said target specific binding ligand is dphenylhydantoin, digoxin or phenobarbital; said immobilized receptor is an antibody which specifically binds to said target specific binding ligand; and wherein

said water-soluble conjugate is formed from said anti-inhibitor antibody and said target specific binding ligand.

16. A dry analytical element comprising a porous spreading layer which contains an immobilized reporter enzyme, said element further comprising an immobilized receptor receptor for a target specific binding ligand.

18. The element of claim 16 wherein said reporter enzyme and receptor are immobilized on polymeric particles within said porous spreading layer.

19. A dry analytical element comprising a nonsporous support having thereon, in order, one or more hydrophilic reagent layers, and a porous spreading layer,

said element further comprising the following reagents wherein said reagents are located in the same or different reagent layers or in the spreading layer:

1) an immobilized receptor for a target specific binding ligand,

2) an immobilized reporter enzyme,

3) an inhibitor antibody having the following characteristics:

a) specifically binds to said reporter enzyme,

b) a dissociation constant less than or equal to about 125 nmolar, and

c) specifically binds to said reporter enzyme in such a manner as to inhibit the enzymatic activity of said reporter enzyme by at least about 80%, and

4) a water soluble conjugate of said target specific binding ligand and an anti-

inhibitor antibody having the following characteristics:

a) specifically binds to said reporter enzyme,

b) a dissociation constant less than or equal to about 50 nmolar, and

c) specifically binds to said reporter enzyme in such a manner that the enzymatic activity of said reporter enzyme is diminished by no more than 20%, and wherein said anti-inhibitor antibody prevents said inhibitor antibody from binding with said said reporter enzyme,

provided that reagents 1) and 2) are kept separated from reagents 3) and 4) but are available for reaction when said element is used in an assay for said target specific binding ligand.

☐ 12. Document ID: US 5221736 A

L22: Entry 12 of 13 File: USPT Jun 22, 1993

DOCUMENT-IDENTIFIER: US 5221736 A
**** See image for Certificate of Correction ****
TITLE: Sequential peptide and oligonucleotide syntheses using immunoaffinity techniques

CLAIMS:

1. A method for the synthesis of an oligonucleotide comprising:

(a) preparing a 3'-blocked nucleotide compound of at least one nucleotide in length;

(b) reacting a 3'-activated, 5'-blocked nucleotide with the 3'-blocked nucleotide compound to produce a mixture of an extended nucleotide compound and an unreacted nucleotide compound;

(c) adding a 5'-terminus capping agent to the mixture to cap the unreacted nucleotide compound;

(d) combining the mixture of the extended nucleotide compound and the 5'-capped unreacted nucleotide compound with an affinity agent that is selective for the cap on the 5'-capped unreacted nucleotide compound; and

(e) isolating the extended nucleotide compound thereby producing the oligonucleotide.

2. A method for the synthesis of an oligonucleotide comprising:

conducting a series of reactions which couple together the nucleotides of the oligonucleotide;

at the completion of each coupling reaction, capping the 5'-terminus of any single

or multi nucleotide-like side product that did not undergo the coupling reaction, thereby producing 5'-capped side products; and

removing the 5'-capped side products by their binding with affinity agents that are selective for the 5'-cap on the side products.

3. A method for the synthesis of an comprising:

(a) preparing a 3'-blocked nucleotide compound of at least one nucleotide in length;

(b) reacting a 3'-activated, 5'-blocked nucleotide with the 3'-blocked nucleotide compound to produce a mixture of a 5'-blocked extended nucleotide compound and an unreacted nucleotide compound, wherein the 5'-blocking agent is derived from a first 5'-terminus capping agent;

(c) adding an second 5'-terminus capping agent to the mixture to cap the unreacted nucleotide compound, the second capping agent being different from the first capping agent;

(d) combining the mixture of 5'-blocked extended nucleotide compound and the second-capped unreacted nucleotide compound with an affinity agent that is selective for the 5'-block on the capped extended nucleotide compound; and

(e) isolating the extended nucleotide compound thereby producing the oligonucleotide.

4. A method for the synthesis of an oligonucleotide comprising:

conducting a series of reactions which couple together the nucleotides of the oligonucleotide;

at the completion of each coupling reaction, capping the 5'-terminus of any single or multi nucleotide-like side product that did not undergo the coupling reaction with a side product capping agent, thereby producing 5'-capped side products; and

in the final nucleotide coupling reaction to form the desired oligonucleotide, reacting a 5'-capped nucleotide with the extended chain nucleotide compound to form the 5'-capped desired oligonucleotide, the 5'-cap of the 5'-capped desired nucleotide being different from the 5'-cap of the 5'-capped side products;

isolating the 5'-capped desired oligonucleotide by its binding with an affinity agent that is selective for the 5'-cap on the 5'-capped desired oligonucleotide.

5. The method of claim 1 further comprising:

repeating steps (a) through (c) one or more times;

removing the 5'-blocked group of the extended nucleotide compound after repetition of step (c) to form a new 3'-blocked nucleotide compound for each repetition of step (a); and,

reacting the same or a different 3'-activated, 5'-blocked nucleotide at step (b).

6. The method of claim 1 wherein the 5'-terminus terminus capping agents, and their corresponding affinity agents constitute affinity pairs selected from the group consisting of an antigenic capping agent with an antibody, an enzymatic substrate, inhibitor or cofactor capping agent with an enzyme, a vitamin or sugar capping agent agent with an apoenzyme, and a covalent bond capping agent with an covalent bond forming reactant.

7. The method of claim 6 wherein the affinity pair is an antigenic capping agent and and an antibody, and the antigenic capping agent is an acylating, phosphorylating or carbamylating agent that reacts with hydroxyl groups.

8. The method of claim 7 wherein the antigenic hydroxyl capping agent is a substituted or unsubstituted aromatic isocyanate, a dialkyltriazoylphosphine, a dialkyl or diaryl phosphoramidate, an aliphatic acid halide of 2 to 10 carbons or a substituted or unsubstituted phthalic anhydride, benzoyl halide or naphthoyl halide, the substituents being selected from the group consisting of mono-, di- or tri-nitro; mono-, di- or tri-methoxy; mono-, di- or tri-cyano; mono-, di- or tri-carboxy; or mono-azido; and the halide being fluoro, chloro, bromo and iodo.

9. The method of claim 6 wherein the affinity pair is an antigenic capping agent and and an antibody, and the antibodies are monoclonal antibodies, polyclonal antibodies or antibody fragments of monoclonal or polyclonal antibodies.

10. The method of claim 9 wherein antibody fragments are used, and are selected from from the group consisting of Fab, Fab.sub.2, light or heavy chains, light or heavy chain fragments, recombinant variable region fragments, and dAbs antibody fragments.

11. The method of claim 6, wherein the affinity pair is the enzymatic substrate, inhibitor or cofactor N-terminus capping agent and the complementary enzyme affinity affinity agent, which pair is selected from the group consisting of aromatic or heteroaromatic sulfanilamide or sulfanilic acid derivatives with carbonic anhydrase, lipoic acid and its derivatives with lipoamide dehydrogenase, and anthranilic acid or its derivatives with anthranilate synthetase complex or its component enzyme anthranilate-5-phosphoribosyl pyrophosphate phosphoribosyl transferase.

12. The method of claim 6 wherein the affinity pair is the vitamin or sugar N-terminus capping agent and the complementary apoenzyme or lectin affinity agent, which pair is selected from the group consisting of NAD with a protein alcohol dehydrogenase, 4-methyl pyrazol with an alcohol dehydrogenase, riboflavin with a glucose oxidase, lipoic acid with a lipamide dehydrogenase, thiamine pyrophospate with thiamine binding protein from E. coli, and biotin with avidin or strepavidin.

13. The method of claim 6 wherein the a finity pair is the covalent bond forming, N-N-terminus capping agent and the complementary covalent bond reactant affinity agent, which pair is selected from the group consisting of acrylic acid or its derivatives with a diene or acrylamide derivative; p-vinylbenzoic acid or its derivatives with a copolymer; and a photoreactive compound selected from the group consisting of benzophenone phenylalanine, p-benzophenone N-benzylphenylalanine, p-azidobenzoic acid, p-azidobenzoyl chloride, p-azidobenzoylglycine, 3-azido-5-nitrobenzoic acid, 3-azido-5-nitrobenzoylglycine, an aromatic azido compound containing a carboxylic side chain, N-hydroxysuccinate ester of N-ethyl-maleimide and m-maleimidobenzoyl-N-hyroxylsulfoxysuccinimide ester with a photolabile affinity agent; and wherein the respective reactions are accomplished by a Diels-Alder reacton, a free radical mechanism, or a photolytic mechanism at a predetermined wavelength of light respectively.

14. The method of claim 1 wherein the 5'-terminus capping agent displays both affinity and photolabile properties, and wherein the mixture of 5'-capped unreacted nucleotide and extended nucleotide are first combined with an affinity agent that selectively binds with said photolabile portion of said 5'-capping agent in the presence of a predetermined wavelength of light, thereby separating the bulk of the 5'-capped unreacted nucleotide from the extended nucleotide, and further wherein the resulting mixture is next combined with an affinity agent that is specific for said 5'-capping agent to separate the remaining 5'-capped unreacted nucleotide from

the extended nucleotide.

15. The method of claim 1 further comprising:

adding a chaotropic agent to the mixture containing the 5'-capped oligonucleotides after the capping step, but before combining the capped oligonucleotides with their complementary affinity agents; and

diluting the mixture containing the chaotropic agent and 5'-capped oligonucleotides before binding them with their respective affinity agents.

16. The method of claim 15 wherein the chaotropic agent is selected from the group consisting of urea, guanidine hydrochloride or formamide.

17. The method of claim 1 further comprising adding an anhydrous organic solvent to the mixture containing 5'-capped oligonucleotides after the capping step, but before combining the capped oligonucleotides with their complementary affinity agents, wherein the 5'-capped oligonucleotides are solubilized.

18. The method of claim 17 wherein the anhydrous organic solvent is selected from the group consisting of dimethyl formamide, dimethyl sulfoxide, dioxin, acetonitrile, ethanol, methanol, acetone, dimethylglycol and methylethylglycol.

19. A method according to claim 3 further comprising:

at step (d), sequentially combining the 5'-blocked extended nucleotide compound and the second-capped unreacted nucleotide compound with two different affinity agents that are selective for the 5'-block on the capped extended nucleotide compound and second-cap on the unreacted nucleotide compound respectively.

20. A method for synthesis of an oligonucleotide, which comprises:

(a) preparing a 3'-blocked nucleotide compound of at least one nucleotide in length;

(b) reacting a 3'-activated, 5'-blocked nucleotide with the 3'-blocked nucleotide compound to produce a mixture of an extended nucleotide compound and an unreacted nucleotide compound;

(c) adding an antigenic hydroxyl capping agent to the mixture to antigenically cap the unreacted nucleotide compound;

(d) combining the extended nucleotide compound and the antigenically capped unreacted nucleotide compound with antibodies that are immunoselective for the antigenic cap; and

(e) isolating the extended nucleotide compound thereby producing the oligonucleotide.

21. A method according to claim 20, further comprising:

repeating steps (a) through (c) one or more times;

removing the 5'-blocking group of the extended nucleotide compound after repetition of step (c) to form a new 3'-blocked nucleotide compound for each repetition of step (a); and

reacting the same or a different 3'-activated, 5'-blocked nucleotide at step (b).

22. A method for the synthesis of an oligonucleotide, which comprises:

(a) preparing a 3'-blocked nucleotide compound of at least one nucleotide in length; length;

(b) reacting a 3'-activated, 5'-antigenically capped nucleotide with the 3' blocked nucleotide compound to produce a mixture of a 5'-antigenically capped, extended nucleotide compound and an unreacted nucleotide compound;

(c) reacting the mixture with an acyl agent to convert the unreacted nucleotide compound into a 5'-acyl derivative;

(d) combining the mixture with antibodies, which are immunoselective for the antigenic cap, and isolating the 5'-antigenically capped, extended nucleotide compound; and

(e) removing the antigenic cap from the 5'-antigenically capped, extended nucleotide nucleotide compound, thereby producing the oligonucleotide.

23. A method according to claim 20, further comprising:

repeating steps (a) through (c) one or more times;

after each repetition of step (c) removing the 5'-antigenic cap from the 5'-antigenically capped extended nucleotide compound to form a new 3'-blocked nucleotide compound for use in each repetition of step (a); and,

reacting the same or a different 3'-activated, 5'-antigenically capped nucleotide at each repetition of step (b).

24. A method for the synthesis of an oligonucleotide, which comprises:

conducting a series of reactions which couple together the nucleotides of the oligonucleotide;

at the completion of each coupling reaction, antigenically capping the 5'-hydroxyl terminus of any single or multinucleotide-like side product that did not undergo the coupling reaction, thereby producing antigenically capped side products; and,

removing the antigenically capped side products by their conjugation with antibodies that are immunospecific for the antigenic cap.

25. A method according to claim 24, wherein the removing step is performed at the end for the series of coupling reactions.

26. A method for separating a synthesized mixture of a 5'-blocked oligonucleotide and 5'-unblocked single or multinucleotide-like side products, which comprises:

antigenically capping the 5'-hydroxy termini of the single or multinucleotide-like side products with a hydroxyl reactive antigenic capped agent to form antigenically capped side products, and conjugating the antigenically capped side products with antibodies that are immunospecific for the antigenic cap.

27. A method for separating a synthesized mixture of a 5'-blocked oligonucleotide and 5'-unblocked single or multinucleotide-like side products which comprises:

capping the 5'-hydroxyl termini of the single or multinucleotide-like side products with a hydroxyl reactive capping agent, employing dimethyltrityl, dansyl or another antigenic capping agent for failed single or multinucleotides as the 5'-block of the 5'-blocked oligonucleotide, said hydroxyl reactive capping agent and said dimethyltrityl, dansyl or antigenic capping agent being different; and

conjugating the resulting mixture with first antibodies that are immunospecific for the dimethyl trityl, dansyl or antigenic capping agent.

28. A method according to claim 27 further comprising employing a second antigenic capping agent as the hydroxyl reactive capping agent and, in addition to conjugating with the first antibodies, further conjugating the resulting mixture with second antibodies that are immunospecific for the second antigenic capping agent.

29. A method according to claim 20, 21, 22, 23, 24, 25, 26 or 27 wherein the antigenic capping agent is an acylating, phosphorylating or carbamylating agent that reacts with hydroxyl groups.

30. A method according to claim 29, wherein the antigenic capping agent is a substituted or unsubstituted aromatic isocyanate, dialkyltriazoylphosphine, an aliphatic acid halide of 2 to 10 carbons, or a substituted or unsubstituted phthalic anhydride, benzoyl halide or naphthoyl halide, the substituents being selected from the group consisting of mono-, di- or tri-nitro; mono-, di- or tri-methoxy; mono-, di- or tri-cyano; and mono-, di-, or tri-carboxy and the halide being fluoro, chloro, bromo or iodo.

31. A method according to claim 20, 22, 24, 26 or 27 wherein the antibodies are polyclonal.

32. A method according to claim 20, 22, 24, 26 or 27 wherein the antibodies are monoclonal.

33. A method according to claim 20, 22, 24, 26 or 27 wherein the antibodies are immobilized.

☐ 13. Document ID: US 4927752 A

L22: Entry 13 of 13 File: USPT May 22, 1990

DOCUMENT-IDENTIFIER: US 4927752 A
TITLE: Support used in bioluminescent dosing of enzymes, substrates or enzymatic inhibitors

CLAIMS:

1. An immobilized enzyme device, comprising:

a transparent hydrophobic plastic support;

a layer of an amino acid polymer coating the support, said amino acid polymer having hydrophobic portions and hydrophilic portions and said amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the support and the hydrophilic portions of the polymer extend outwardly from the support; and

an enzyme composition adsorbed to the hydrophilic portions of the polymer, said composition comprising a luciferase.

7. The device of claim 1 wherein the enzyme composition further comprises a dehydrogenase or a kinase.

8. A method for making an immobilized enzyme device comprising:

coating a transparent hydrophobic plastic support with a layer of an amino acid polymer to form a pretreated support, said amino acid polymer having hydrophobic portions and having hydrophilic portions and amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the hydrophobic plastic support and the hydrophilic portions of the polymer extend outwardly from the plastic support;

immobilizing an enzyme composition on the precoated support by adsorbing the enzyme composition to the hydrophilic portions of the amino acid polymer, said enzyme composition comprising a luciferase.

9. The method of claim 8, further comprising the step of lyophilizing the immobilized enzyme composition.

10. An immobilized enzyme device made by the process of claim 8.

11. The method of claim 8, wherein the transparent hydrophobic plastic substrate comprises polystyrene.

12. The method of claim 8, wherein the amino acid polymer comprises polylysine.

13. The method of claim 8, wherein the amino acid polymer comprises a copolymer of polylysine, polyarganine or polyhistidine and a hydrophobic amino acid polymer.

14. The method of claim 13, wherein the amino acid polymer comprises a copolymer of polylysine and polyphenylalanine.

15. The method of claim 8, wherein the enzyme composition further comprises NADH:FMN NADH:FMN oxidoreductase or NADPH:FMN oxidoreductase.

16. The method of claim 8, wherein the enzyme composition further comprises a dehydrogenase or a kinase.

17. A method for determining a test enzyme in a test solution, comprising:

contacting an immobilized enzyme device with a test solution,

said immobilized enzyme device comprises:

a transparent hydrophobic plastic support;

a layer of an amino acid polymer coating the support, said amino acid polymer having hydrophobic portions and hydrophilic portions and said amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the support and the hydrophilic portions of the polymer extend outwardly from the support; and

an enzyme composition adsorbed to the hydrophilic portions of the polymer, said composition comprising a luciferase; and

said test solution comprising:

the test enzyme and chemical species that enzymatically react with the test enzyme and the luciferase to emit photons;

detecting photoemission from the contacted test solution; and

quantifying the photoemission to determine the test enzyme in the test solution.

18. The method of claim 17, wherein the chemical species comprise a substrate of the the test enzyme, a substrate of the luciferase and ADP.

19. The method of claim 17, wherein the test enzyme is a kinase.

20. The method of claim 17, wherein the enzyme composition further comprises NADH:FMN oxidoreductase or NADPH:FMN oxidoreductase.

21. The method of claim 20, wherein the chemical species comprise a substrate of the the test enzyme, a substrate of the luciferase, FMN and NAD or NADP.

22. The method of claim 17, wherein the test enzyme is a dehydrogenase.

23. The method of claim 17, wherein the test enzyme is conjugated with a protein and and determining the test enzyme allows determination of the protein.

24. The method of claim 17, wherein the test enzyme is conjugated with an antibody and determining the test enzyme allows determination of the antibody.

25. A method for determining a substrate of a test enzyme, comprising:

contacting an immobilized enzyme device with a test solution,

said immobilized enzyme device comprising:

a transparent hydrophobic plastic support;

a layer of an amino acid polymer coating the support, said amino acid polymer having hydrophobic portions and hydrophilic portions and said amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the support and the hydrophilic portions of the polymer extend outwardly from the support; and

an enzyme composition adsorbed to the hydrophilic portions of the polymer, said composition comprising a luciferase and the test enzyme; and said test solution comprising:

the substrate of the test enzyme, and chemical species that enzymatically react with the test enzyme, and the luciferase to emit photons;

detecting the photoemission from the contacted test solution; and

quantifying the photoemission to determine the substrate of the test enzyme.

26. The method of claim 25, wherein the chemical species comprise a substrate for the luciferase and ADP.

27. The method of claim 25, wherein the enzyme composition further comprises NADH:FMN oxidoreductase or NADPH:FMN oxidoreductase.

28. The method of claim 25, wherein the chemical species comprise a substrate of the the luciferase, FMN and NAD or NADP.

29. A method for determining an inhibitor of a test enzyme, comprising:

contacting an immobilized enzyme device with a test solution,

said immobilized enzyme device comprises:

a transparent hydrophobic plastic support;

a layer of an amino acid polymer coating the support, said amino acid polymer having hydrophobic portions and hydrophilic portions and said amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the support and the hydrophilic portions of the polymer extend outwardly from the support; and

an enzyme composition adsorbed to the hydrophilic portions of the polymer, said enzyme composition comprising luciferase and the test enzyme, said test solution comprising: the inhibitor of the test enzyme and chemical species that enzymatically enzymatically react with the test enzyme and the luciferase to emit photons;

detecting photoemission from the contacted test solution;

quantifying the photoemission; and

determining the inhibitor by comparing photoemission by the contacted test solution to photoemission by a standard solution comprising said chemical species and a known concentration of said inhibitor.

30. The method of claim 29, wherein the chemical species comprise a substrate of the the luciferase and ADP.

31. The method of claim 29, wherein the enzyme composition further comprises NADH:FMN oxidoreductase or NADPH:FMN oxidoreductase.

32. The method of claim 31, wherein the chemical species further comprise a substrate of the luciferase, FMN and NAD or NADP.

33. A method for determining NADH or NADPH in a test solution, comprising:

contacting an immobilized enzyme device with a test solution;

said immobilized enzyme device comprising:

a transparent hydrophobic plastic support;

a layer of an amino acid polymer coating the support, said amino acid polymer having hydrophobic portions and hydrophilic portions and said amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the support and the hydrophilic portions of the polymer extend outwardly from the support; and

an enzyme composition adsorbed to the hydrophilic portions of the polymer, said composition comprising a luciferase and either NADH:FMN oxidoreductase or NADPH:FMN oxidoreductase; and

said test solution comprising:

NADH or NADPH, FMN and a substrate for the luciferase; and

so as to emit photons detecting photoemission from the contacted test solution; and

quantifying the photoemission to determine the NADH or NADPH in the test solution.

34. A method for determining ATP in a test solution, comprising:

contacting an immobilized enzyme device with a test solution,

said immobilized enzyme device comprising:

a transparent hydrophobic plastic support;

a layer of an amino acid polymer coating the support, said amino acid polymer having hydrophobic portions and hydrophilic portions and said amino acid polymer being oriented so that the hydrophobic portions of the polymer are bound to the support and the hydrophilic portions of the polymer extend outwardly from the support; and

an enzyme composition adsorbed to the hydrophilic portions of the polymer, said composition comprising a luciferase; and

said test solution comprising ATP and a substrate for the luciferase so as to emit photons,

detecting photoemission from the contacted test solution; and

quantifying the photoemission to determine the ATP in test solution.

Search Results - Record(s) 1 through 12 of 12 returned.

L23: Entry 1 of 12 File: USPT Mar 23, 2004

US-PAT-NO: 6710026
DOCUMENT-IDENTIFIER: US 6710026 B1

TITLE: Protein domains in the hepatic glycogen-targetting subunit of protein phosphatase 1 and methods of making and using the same

DATE-ISSUED: March 23, 2004

US-CL-CURRENT: 514/2; 436/86, 530/300

INT-CL: [07] A61 K 38/00, C07 K 7/00, G01 N 33/00

L23: Entry 2 of 12 File: USPT Jan 27, 2004

US-PAT-NO: 6682898
DOCUMENT-IDENTIFIER: US 6682898 B2

TITLE: High-throughput screening assays for modulators of STAT4 activity

DATE-ISSUED: January 27, 2004

US-CL-CURRENT: 435/7.1; 435/7.8, 436/501, 436/518, 436/537, 436/540, 436/542

INT-CL: [07] G01 N 33/53, G01 N 33/566

L23: Entry 3 of 12 File: USPT Dec 23, 2003

US-PAT-NO: 6667179
DOCUMENT-IDENTIFIER: US 6667179 B1

TITLE: Semiconductor luminescence quenchers for detecting proximal molecular binding events

DATE-ISSUED: December 23, 2003

US-CL-CURRENT: 436/517; 435/6, 435/7.1, 436/164, 436/165, 436/172, 436/523, 436/527, 436/537, 436/546, 436/805

INT-CL: [07] G01 N 33/557, G01 N 33/53

L23: Entry 4 of 12 File: USPT Apr 15, 2003

US-PAT-NO: 6548266
DOCUMENT-IDENTIFIER: US 6548266 B1

TITLE: Assay for detecting the enzymatic activity of a phosphorylation enzyme using enhanced signal generation

DATE-ISSUED: April 15, 2003

US-CL-CURRENT: 435/15; 435/174, 435/183, 435/21, 435/23, 435/24, 435/4, 435/7.1

INT-CL: [07] C12 Q 1/48, C12 Q 1/42, C12 Q 1/37, C12 Q 1/00

L23: Entry 5 of 12 File: USPT Feb 4, 2003

US-PAT-NO: 6514689
DOCUMENT-IDENTIFIER: US 6514689 B2

TITLE: Hydrogel biosensor

DATE-ISSUED: February 4, 2003

US-CL-CURRENT: 435/4; 435/287.1, 435/287.7, 604/891.1, 604/892.1

INT-CL: [07] C12 Q 1/00, C12 M 1/34, A61 K 9/22

L23: Entry 6 of 12 File: USPT Jun 26, 2001

US-PAT-NO: 6251621
DOCUMENT-IDENTIFIER: US 6251621 B1

TITLE: Reporter enzyme release technology: methods of assaying for the presence of aspartic proteases and other hydrolytic enzyme activities

DATE-ISSUED: June 26, 2001

US-CL-CURRENT: 435/18

INT-CL: [07] C12 Q 1/34

L23: Entry 7 of 12 File: USPT Mar 27, 2001

US-PAT-NO: 6207391
DOCUMENT-IDENTIFIER: US 6207391 B1

TITLE: High-throughput screening assays for modulators of STAT4 and STAT6 activity

DATE-ISSUED: March 27, 2001

US-CL-CURRENT: 435/7.1

INT-CL: [07] G01 N 33/53

L23: Entry 8 of 12 File: USPT Jun 13, 2000

US-PAT-NO: 6074852
DOCUMENT-IDENTIFIER: US 6074852 A

TITLE: Hepatitis C virus asialoglycoproteins

DATE-ISSUED: June 13, 2000

US-CL-CURRENT: 435/69.9; 424/185.1, 424/228.1, 435/5, 530/395, 530/826

INT-CL: [07] C12 Q 1/70, C12 P 21/04, A61 K 39/29

L23: Entry 9 of 12 File: USPT Nov 10, 1998

US-PAT-NO: 5834216
DOCUMENT-IDENTIFIER: US 5834216 A

TITLE: Screening methods for the identification of inducers and inhibitors of programmed cell death (apoptosis)

DATE-ISSUED: November 10, 1998

US-CL-CURRENT: 435/7.21; 435/15, 436/516

INT-CL: [06] G01 N 33/53, G01 N 3/561, C12 Q 1/12

L23: Entry 10 of 12 File: USPT Apr 28, 1998

US-PAT-NO: 5744313
DOCUMENT-IDENTIFIER: US 5744313 A

TITLE: Assay employing novel protein domain which binds tyrosine phosphorylated proteins

DATE-ISSUED: April 28, 1998

US-CL-CURRENT: 435/7.1; 435/6, 435/7.2, 436/501

INT-CL: [06] G01 N 33/53

L23: Entry 11 of 12 File: USPT Oct 15, 1996

US-PAT-NO: 5565326
DOCUMENT-IDENTIFIER: US 5565326 A

TITLE: Separation-free specific binding assays using anti-inhibitor antibodies

DATE-ISSUED: October 15, 1996

US-CL-CURRENT: 435/7.71; 435/962, 435/969, 435/970, 435/975, 436/518, 436/523, 436/529, 436/530, 436/531, 436/548, 436/816, 436/822

INT-CL: [06] G01 N 33/53

L23: Entry 12 of 12 File: USPT Jun 22, 1993

US-PAT-NO: 5221736
DOCUMENT-IDENTIFIER: US 5221736 A
**** See image for Certificate of Correction ****

TITLE: Sequential peptide and oligonucleotide syntheses using immunoaffinity

techniques

DATE-ISSUED: June 22, 1993

US-CL-CURRENT: 536/25.31; 435/4, 435/5, 435/6, 435/7.5, 435/7.8, 435/803, 435/810, 435/91.5, 436/518, 436/531, 436/824, 530/387.1, 536/26.71

INT-CL: [05] C07H 21/00

Previous Page Next Page